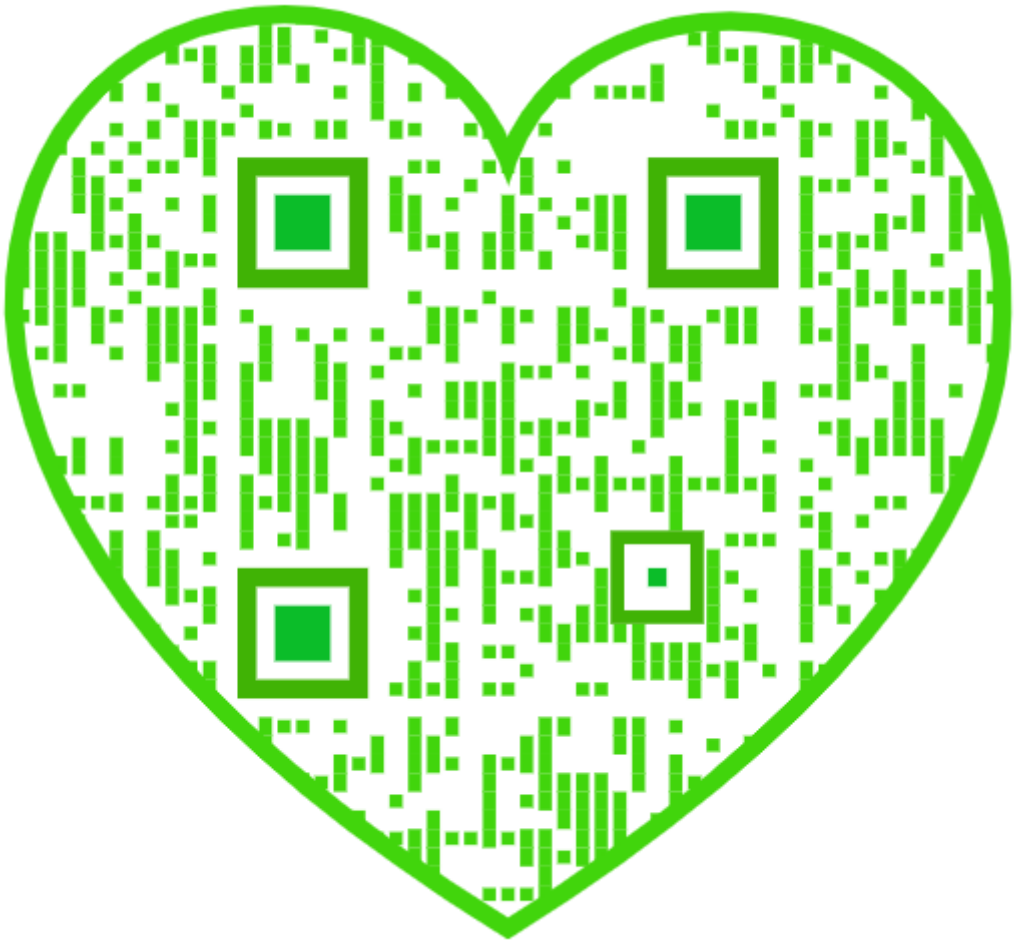

# A TEXT-BOOK

OF

# URINE ANALYSIS

FOR

## STUDENTS AND PRACTITIONERS OF MEDICINE.

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BY

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*WITH NUMEROUS ILLUSTRATIONS.*

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# PREFACE

In the following pages the subject of practical urine analysis is presented in a concise manner adapted to the requirements of the medical student in systematic class work, and also to the wants of the medical practitioner, who has occasion to make something more than the usual simple qualitative tests in urine examinations. A considerable number of quantitative methods are carefully described, and in such a manner, it is believed, that any one with a slight previous training in the manipulation of chemical apparatus can follow them. On the other hand methods of doubtful applicability are consistently excluded.

This book contains much of the matter which appeared some years ago in the author's "Chemical Physiology and Urine Analysis." That work being out of print, the chapters on urine analysis have been rewritten and enlarged to appear, with additional chapters, in the present form. Two chapters are devoted to the microscopic examination of sediments.

While the book is essentially one of analysis, not of diagnosis, numerous references are made throughout the text to the clinical significance of what is found by the various tests, and in the appendix a section is devoted to a tabular statement of the relation of pathological condi-

tions to the chemical composition of the urine. For many valuable suggestions in this direction the author is indebted to his colleague, Dr. Frank S. Johnson. He must also acknowledge the assistance of Dr. Frank X. Walls in the preparation of illustrations, and of Mr. Frank Wright in the reading of proof.

THE AUTHOR.

*Chicago, March, 1900.*

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# URINE ANALYSIS

## CHAPTER I

### OUTLINE OF TESTS. PRELIMINARY TESTS

The importance of an accurate knowledge of the bodies excreted by the urine has long been recognized and elaborate investigations have been carried out to determine the nature and quantities of these substances, some of which appear normally in health, while others are found only during the progress of disease.

Experiment shows that normally certain products occur in the urine in relatively large amounts, and give to it its prominent characteristics, while of other products the amounts present are so minute that their detection is a matter of no little difficulty.

Certain grave disorders are accompanied by the appearance of certain substances in the urine, and where the chemical or microscopic tests for the latter are simple and unquestionably correct we have at hand a convenient aid to diagnosis. In many cases, however, it is true that we are unable to trace the relation between small amounts of substances occasionally appearing in urine and any specific disorder or condition of the body. The detection of such substances is naturally without value in diagnosis, at the present time.

Yet it would be unwise to neglect the study of such traces because, as medical science progresses, new relations are from time to time brought to light which give value to data which at one time may have been considered wholly unimportant. Complete handbooks on the urine give prominence to many topics which will not be touched upon in what follows because we are here concerned with phenomena, everywhere recognized as important and the bearings of which, in the main at least, are understood.

In the practical analysis of urine, such as is customary for clinical purposes, comparatively few tests are required, and little apparatus is necessary beyond that already used for other examinations. Frequently a single test is sufficient to determine all the physician needs to know; for instance, regarding the presence or absence of sugar or albumin.

In the following pages those tests and processes will be described which have been shown by experience to be amply sufficient for all practical requirements. Some of these are qualitative, others quantitative, and may be tabulated as follows:

Qualitative tests.

1. Observation of color and odor.
2. The reaction, whether acid or alkaline.
3. The tests for albumin.
4. The tests for sugar.
5. The tests for the characteristic biliary acids and coloring-matters.
6. The various tests for blood.
7. Tests for other coloring-matters.
8. The examination of the sediment.

**Quantitative tests.**

9. Determination of specific gravity.
10. " " the amount of albumin.
11. " " " " sugar.
12. " " " " uric acid.
13. " " " " urea.
14. " " " " phosphates.
15. " " " " chlorides.

The above includes the usual and important tests. A few others will be given in the proper place; for instance, tests for acetone and diacetic acid, which under circumstances may have importance.

Normally, urine contains as its most important constituents urea, sodium chloride, certain phosphates and urates, and smaller amounts of other substances as hippuric acid, xanthin, creatinin, traces of phenols, etc.

Several writers have given the results of complete urine analyses in tabular form. These results are not very concordant, as might be expected from the character of urine itself. Two tables often quoted will be given here for comparison.

According to Thudichum the average volume of urine passed per day is 1400 to 1600 cc., and the solid matter contained in the daily excretion he gives as follows, for a man weighing about 140 pounds.

| | Grams. |
|---|---|
| Urea | 30-40 |
| Uric acid | 0.50 |
| Creatin and creatinin | 0.75 |
| Hippuric acid | 0.50 |
| Cryptophanic acid | 0.65 |
| Biliary acids | 0.012 |
| Acetic acid | 0.288 |

| | Grams. |
|---|---|
| Formic acid | 0.05 |
| Sulphuric acid, $SO_3$ | 2.00 |
| Other sulphur combinations | 0.20 |
| Alkaline phosphates | 3.66 |
| Earthy phosphates | 1.28 |
| Lime | 0.17 |
| Magnesia | 0.19 |
| Potassium and sodium chlorides | 11.50 |
| Ammonia | 0.70 |

Traces of other bodies are given by Thudichum, but in amounts too small for determination.

Another table given by Parkes presents a better arrangement, and is here given; the figures refer to the amounts excreted in twenty-four hours by a man weighing about 145 pounds.

| | Grams. |
|---|---|
| Urea | 33.18 |
| Uric acid | 0.55 |
| Hippuric acid | 0.40 |
| Creatinin | 0.91 |
| Organic acids and pigments | 10.00 |
| Sulphuric acid, $SO_3$ | 2.01 |
| Phosphoric acid, $P_2O_5$ | 3.16 |
| Calcium | 0.26 |
| Magnesium | 0.21 |
| Potassium | 2.50 |
| Sodium | 11.09 |
| Chlorine | 7.50 |
| Ammonia | 0.77 |

It is evident that the character of the urine depends very largely on the diet, and this is shown in a clear manner by the figures in the following table, which were obtained by Bunge by the analysis of the urine of a healthy man, fed first on a meat diet, and later

on one of wheat bread with salt and butter. Water was freely drunk in both tests.

| | Meat diet. cc. | Bread diet. cc. |
|---|---|---|
| Volume in twenty-four hours. | 1672 | 1920 |
| | Grams. | Grams. |
| Urea | 67.2 | 20.6 |
| Creatinin | 2.163 | 0.961 |
| Uric acid | 1.398 | 0.253 |
| Sulphuric acid, $SO_3$ | 4.674 | 1.265 |
| Phosphoric acid, $P_2O_5$ | 3.437 | 1.658 |
| Lime | 0.328 | 0. 39 |
| Magnesia | 0.294 | 0.139 |
| Potash | 3.308 | 1.314 |
| Soda | 3.991 | 3.923 |
| Chlorine | 3.817 | 4.996 |

These figures represent, of course, extreme cases, but their practical importance is readily recognized.

Pathologically there may appear albumin, sugar, blood, pus, bile pigments and acids, and a number of other bodies insoluble, or of slight solubility, which usually appear as a sediment.

We turn now to an explanation of the various preliminary tests employed.

### Specific Gravity

The density or specific gravity of the urine secreted in twenty-four hours, varies in health between rather wide limits, probably between 1.005 and 1.030. 1.020 may be taken as about the mean value at 15° C.

The specific gravity depends primarily on the amounts of liquid and solid food taken, and on the loss of water from the skin by perspiration. When

this loss is great the specific gravity of the urine is correspondingly increased, other things being equal.

In disease the density may be lowered below or increased above the normal value.

For an absolutely exact determination of the density the use of the pycnometer, Mohr-Westphal balance, or other apparatus is necessary, but for our purpose the *urinometer*, or density bulb, is sufficiently accurate. This little instrument is shown in the adjoining figure. The urine to be tested is poured into a narrow jar, about one cm. wider than the bulb, and after the air bubbles have escaped the urinometer is immersed in it. When it comes to rest the degree at which it stands is read off *below the surface*. Usually the last two figures only of the density are marked on the stem, as 25, instead of 1.025, and these are often given as the density.

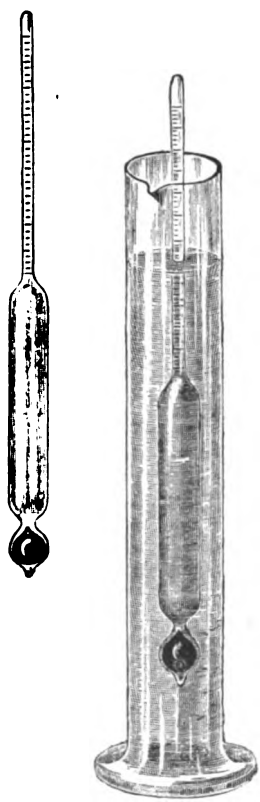

Fig. 1.

As the density of urine decreases about one degree for an increase in temperature of 3° to 5° C. it is important that the test be made at a definite known temperature, as 15° or 25° C. Urinometers have usually been graduated to

give the correct reading at a temperature of 15.5° C. (60° F.). But at the present time we have them for the temperature of 25° C. (77° F.), because this is with us a more common house temperature than the lower one. It is convenient to have the instrument indicate the correct specific gravity without the necessity of cooling. The specific gravity of urine varies approximately as does that of water, with changes of temperature. A table in the appendix shows the rate for water, and by the use of this a correction can be made.

By noting the amount of urine passed in twenty-four hours, and the density of the mixed liquid, a rough determination of the solid matters contained in it can be made. For this purpose it is simply necessary to multiply the last two figures of the density by 2.33 (known as the coefficient of *Haeser*), which gives the approximate number of grams in a liter. By proportion the amount for the day can be calculated from this.

For example, 1,400 cc. of urine was passed, and its density was found to be 1.024.

Then, $24 \times 2.33 = 55.92$,

and, $1{,}000 : 1{,}400 :: 55.92 : x = 78.288$.

This calculation is frequently of service.

As indicated above a variation in the specific gravity of normal urine may be due to several causes, the most important of which are changes in the volume of water drunk or the weight of nitrogenous food and salt digested. The amount of urine excreted daily may be taken as 1,500 cc. in the mean. Assuming

that 15 grams daily is the salt consumption and that it is all excreted with the urine we have through this factor alone a specific gravity of about 1.007. Assuming further that 150 grams of nitrogenous food (considered as pure albumin) are consumed daily and that four-fifths of this amount is daily excreted as urea, the weight of the latter in the urine would be 43 grams. This alone would produce a density of nearly 1.008 and give a percentage composition of 2.84. Combined with the other substances in urine the relative effect of the addition of urea would be greater. All of the solids of the urine have a specific gravity greater than that of water and their presence therefore adds to the specific gravity of the excretion, but changes in the density, due to changes in the amounts of uric acid, phosphates, sulphates, etc., passed, are of less importance because of the relatively small quantities of these substances normally present.

If, with the food consumed normal, the water taken is small in amount, or if a large amount is lost from the skin as perspiration then the density of the excreted urine must be correspondingly higher. A large volume of water consumed or little evaporation from the skin will give a urine of lower density. It is plain, therefore, that great variations in the specific gravity of the urine may occur and from perfectly normal causes.

In disease even greater variations may occur, one of the most characteristic and important being that due to the presence of sugar in *diabetes mellitus*. Here the density may reach 1.040 or higher, while the vol-

ume of urine is above 1,500 or 2,000 cc. in the twenty-four hours. A high specific gravity with *large volume* is always suspicious and suggests presence of dextrose, although occasionally it may be due to presence of large doses of soluble salts taken into the system as remedial agents. A low specific gravity with *small volume* of urine must also call for investigation, as this points to the absence of, or marked decrease in, the normal constituents from some cause. A lower density is observed in diseases where the elimination of urea is slower because of hindered tissue changes, in conditions of malnutrition in general, and in any disease involving the structure of the liver itself. In *acute yellow atrophy* of the liver, for instance, urea is much diminished, and the specific gravity low.

The diminution in excreted chlorides, with normal consumption, in certain diseases is also a factor in causing low specific gravity. This may follow when the salt consumed is eliminated temporarily in various exudations or effusions rather than by the normal channel.

Some of these indications will receive attention later during the discussion of the tests for the common normal and abnormal urine constituents.

## Reaction

In health the reaction of the mixed urine passed through twenty-four hours is always acid.

This normal acid reaction is supposed to be due to the presence of acid phosphates and to small amounts of uric acid and to other free organic acids. The

reaction can be observed by the aid of sensitive litmus paper, but the absolute amount of free acid is very small.

Occasionally urine is passed which gives the so-called *amphoteric* reaction with litmus; that is, it turns blue paper red and red paper blue. It has not been found possible to connect this phenomenon with certainty with any definite pathological condition; it has, therefore, no special clinical significance at the present time. Some hours after a hearty meal an alkaline reaction is frequently observed, giving place soon to the usual acid condition. This alkaline reaction may be due to the presence of small amounts of trisodium phosphate formed during active digestion. The administration of alkaline carbonates, or of certain organic salts, as malates, acetates, tartrates, or citrates, which yield carbonates by final decomposition, may also occasion an alkaline condition. It should be observed that the character of the food consumed has much to do with the quality of the urine as regards acidity or alkalinity. In the consumption of products rich in proteids the oxidation of the sulphur gives rise to sulphuric acid which converts the alkali phosphate of the blood into acid phosphate before excretion. With a diet low in proteids the acidity is greatly decreased. Sometime after it is voided urine always becomes strongly alkaline in reaction, but this change may be delayed for days or weeks even. It is brought about by the decomposition of urea, which is usually a result of bacterial action. In this decomposition ammonium carbonate is formed, the odor of which

often becomes very strong. Anything which prevents or impedes the bacterial activity tends to maintain the ordinary acid or neutral reaction. Salicylic acid, thymol, chloroform, volatile oils, and other antiferments behave in this manner, and are frequently added to specimens of urine to preserve them for investigation. For the preservation of 100 cc. of urine one-fourth of a gram of salicylic acid is enough.

Sometimes the decomposition of the urea takes place in the bladder, the voided urine having then a very marked alkaline reaction and strong odor, usually. Such a change may be brought about by the progress of disease, or may be induced by the introduction of a dirty catheter into the bladder. This carries the organism capable of splitting up the urea, and the condition once established may be maintained for a long time.

Ammonium carbonate results from the reaction. This may be distinguished from the fixed alkalies (hydroxide or carbonate of sodium or potassium) by a very simple test. A piece of sensitive red litmus paper immersed in alkaline urine becomes blue. On drying the paper the color due to the non-volatile alkalies persists, while that of ammonium carbonate disappears. The test has some practical value as it is necessary to distinguish between the alkalinity of urea fermentation and that of an excess of fixed alkali occasionally present. For these tests only fresh sensitive paper can be safely used. The conversion of urea into ammonium carbonate is represented by this equation:

$$CON_2H_4 + 2H_2O = (NH_4)_2CO_3.$$

In highly colored urines it is not always easy to observe the reaction with litmus paper. In this case a method often used in the examination of blood to determine its alkalinity may be applied. This consists in immersing small disks of plaster of Paris in *neutral* litmus solution and then drying them. A few drops of urine are placed on a disk and allowed to remain some minutes. The urine is then washed off leaving a bluish or reddish spot indicating the reaction.

An accurate determination of the acidity of urine can not be readily made, but approximately a value may be obtained by adding a few drops of phenolphthalein to 100 cc. of urine, and then running in tenth-normal sodium hydroxide solution until a permanent pink color is secured. At this stage the acid phosphates of the type $H_2NaPO_4$ are converted into the alkali phosphates of the type $HNa_2PO_4$, and for each cubic centimeter of alkali used 12 milligrams of $H_2NaPO_4$ may be calculated as present. But on account of the color of the urine itself the process is at best somewhat uncertain.

To approximately determine the alkalinity add to 100 cc. of urine a few drops of phenolphthalein, and then tenth-normal sulphuric or hydrochloric acid until the color disappears. If the alkalinity is due to sodium carbonate, for each cc. of the acid used, calculate 5.3 milligrams of $Na_2CO_3$ as present. If due to $Na_3PO_4$ each cc. of alkali used corresponds to 8.2 milligrams. All such tests should be made in a beaker with a wide bottom placed over white paper so

as to disclose a change of color as clearly as possible.

For the preparation of the standard acid and alkali solutions used in these tests see the appendix.

### Odor

The odor of urine is not easily described, as in health it is *sui generis* and characteristic. Normal urine contains traces of complex aromatic bodies, the exact nature of which cannot in all cases be given. These substances are more abundant after a vegetable than after an animal diet, and are especially noteworthy in the urine of persons whose food contains such vegetables as cabbage, radishes, parsnips, asparagus, or the spices. It is well known that certain substances given as remedies give rise to distinct odors in the urine. The administration of turpentine imparts to the urine an odor of violets.

As the odor so largely depends on the nature of the food it may be much modified even in health, and in disease may be characteristically changed. The ammoniacal odor of urea decomposition in the bladder has been referred to, and the peculiar *sweetish* odor of diabetic urine has long been noticed.

But it must be remembered that many strong odors may be developed in the urine soon after passage by the action of ferments other than the *micrococcus urea* which yields ammonium carbonate. In some cases these give rise to what may be called a putrefactive odor.

### Color

The color of urine is described as *straw-yellow.*

Many causes, however, may produce a change in this shade, leaving the urine still normal. As can be readily seen the color is closely dependent on concentration and must, therefore, vary with the amount of liquid taken into the stomach.

Certain foods from the vegetable kingdom possess characteristic coloring-matters which pass, more or less changed, into the urine. As long as the latter is acid the presence of these may not be noticed, but with a change of reaction a change of color may follow, usually to reddish.

Santonin imparts a yellowish color to urine, reddened by alkalies. In pathological conditions the color of urine is often characteristic and of great importance in diagnosis. The presence of blood, for instance, is indicated by a more or less sharp shade of red, bile by a peculiar greenish brown, especially noticeable in froth produced on shaking. The urine of *diabetes mellitus* is generally very pale, while the urine of fevers is usually highly colored not only from the diminution of water, but also from the presence of abnormal coloring-matters. Different shades are produced by the presence of *altered* blood and bile constituents, which will be referred to later. The real color of the urine is often obscured by loss of transparency due to precipitation. Normal urine is generally perfectly transparent when passed, but sometimes cloudy from presence of a suspended precipitate of mucus or phosphates. On becoming alkaline a precipitation of earthy phosphates usually follows. A precipitate of urates, without change of reaction, often

takes place by simply lowering the temperature of the urine. This precipitate, however, disappears on the application of a slight heat to the urine, and leaves the latter clear for examination of color.

Clinically, the color indications of greatest importance are those due to the presence of derivatives from the blood or bile. These may be unaltered elements of the blood or bile or decomposition products of their essential coloring-matters.

In a following section on the tests for abnormal coloring-matters in urine this question will be again taken up.

# CHAPTER II

## THE TESTS FOR ALBUMINS

Albuminous bodies do not occur in normal urine except, perhaps, in mere traces. Numerous investigations have been published on this subject, and while some of the recent ones would seem to show the probability of a *physiological albuminuria*, others, seemingly as thorough, lead to quite the opposite conclusion.

Temporarily, it is true, albumin may be found in the urine of healthy individuals, as after the consumption of large quantities of egg albumin, or after the action of some cause producing a sudden alteration of the blood pressure, but the amounts found in such cases are too small, and their occurrence too rare, to permit them to be classed as anything but accidental. It is certain that the presence of any appreciable amount of albumin in the urine and the persistence of the same must be looked upon as a *pathological* phenomenon and one of the greatest importance to the physician.

Albumins may appear in urine from several sources, most frequently, probably, because of some structural change in the tubules of the kidneys which permits a filtration from the blood. But this is not always the case as they may appear from sources in no way dependent on renal disorder, from lesions of the ureters,

bladder, or urethra, for instance, in which case blood or pus may be present. Ordinary serum albumin is the usual, but not the only proteid body which may appear in urine. Half a dozen or more modifications have been described as occurring under different circumstances, but the evidence for some of these appears to be of doubtful character. Certain forms are not readily detected or identified. In what follows, tests will be given for those proteid bodies which can be detected with certainty and whose presence has some definite clinical importance.

### 1. Serum Albumin

The presence of serum albumin in the urine is a characteristic of what is ordinarily termed *albuminuria.* As intimated above albuminous bodies may appear in the urine from different sources. The presence of serum albumin suggests (*a*) a functional or structural disorder of some part of the essential tissue of the kidney, in which case we have *renal albuminuria* or *true albuminuria*, or (*b*) a lesion of some part of the urinary tract below the kidney, in which case we have what is called *false* or *accidental albuminuria.*

Renal albuminuria is the condition appearing in Bright's disease or *acute parenchymatous nephritis* and in other pathological conditions in which a change of the diffusion membrane is involved. It is also frequently induced by derangements in the circulation due to heart diseases, high fevers, etc., which in turn may react and give rise to a derangement of the kidney itself. That is to say, the causes producing cer-

tain febrile conditions may extend to the structure of the renal filtering apparatus and so alter its condition that the passage of albumin is no longer hindered but becomes continuous.

Under all such circumstances the albumin passing through the kidney is generally accompanied with something which suggests its origin. There may be here an excessive amount of the epithelial lining of the tubules, or plugs of coagulated albumin, mucus, or of the wax-like, partially degenerated albumin known as lardacein, all in the form of "casts" of the uriniferous tubules. These may be readily seen and recognized by the microscope.

As intimated above, false or accidental albuminuria can originate from several causes and in general is a condition of far less clinical importance than the other. It is usually possible to determine by a few examinations the real nature of the disorder by aid of the facts just mentioned.

Because of the very great importance of the subject to the physician, much attention has been given to the question of albumin tests, and the number of reactions proposed for its detection reach, possibly, the hundreds. Many of these are of such extreme delicacy and so easy of execution that to make a choice of a few is by no means a simple matter.

The best of them depend on the fact that the soluble serum albumin, which finds its way into the urine, can be coagulated and made visible as white flocculi, or as a white cloud when present in small quantity. Of the various methods of producing this coagulation,

only those will be mentioned which are most characteristic, and practically the most useful.

## Qualitative Coagulation Tests

**Coagulation by Heat.**—When a sample of urine is boiled, a precipitate usually forms. This in most cases consists of *earthy phosphates*, and is often sufficient to conceal a precipitation of albumin possibly present. If now to the boiled sample about one-tenth its volume of strong nitric acid be added, the precipitated phosphates will disappear, while the albumin will remain coagulated. It is necessary to add as much nitric acid as is here indicated, *because a small amount may sometimes dissolve coagulated albumin, forming soluble acid-albumin.* This acid-albumin is broken up on the addition of more acid.

Even when boiling does not throw down a precipitate, the addition of nitric acid cannot be omitted, as under certain circumstances the heating may produce a soluble combination between alkalies present and albumin, which is stable. Nitric acid in sufficient quantity will break up this combination and bring about coagulation.

Under most circumstances this heat test, as outlined, is sufficient, and the possibility of making a mistake is very small. It is shown in works on chemical physiology that small amounts of albumin combine readily with weak acids and alkalies, forming soluble and stable combinations known as *acid-albumin* and *alkali-albumin.*

If the urine has a neutral or alkaline reaction

to begin with a small amount of alkali-albumin would escape detection by heating alone. On addition of just the proper amount of acetic acid to neutralize the alkali, the application of heat will cause a coagulation, but a *slight* excess of this acid might convert the *alkali-albumin* into *acid-albumin*, equally hard to precipitate. Traces of nitric acid, and in a marked degree hydrochloric acid, behave in the same manner, but the addition of larger amounts of nitric acid is free from this objection because in proper amount this acid is able to decompose both acid- and alkali-albumin.

When taken for examination, urine is frequently cloudy from the presence of precipitated urates or earthy phosphates. Heat is sufficient to dissipate the cloud if due to the urates, but the phosphate cloud is rendered heavier. It is always a good plan to carefully filter the urine, if in the least degree turbid, before undertaking the test.

With old samples of urine which have undergone the urea fermentation and have become alkaline, the test by heat and subsequent addition of acid is not always satisfactory or convenient. In such cases it is best to proceed at once to a method which disposes of the excess of alkali at the start, and in such a manner as to cause no confusion.

**Coagulation by Nitric Acid.**—As indicated above nitric acid can coagulate albumin, and this test is frequently employed without previous boiling. When applied to fresh urine the test may be made in this manner.

Several cc. of the strong acid are warmed in a test-

tube, and over this is carefully poured an equal volume of urine, so as to overlie without mixing. If albumin is present a white ring appears at the surface between the two liquids. When the urine contains an excess of coloring-matter the ring is variously tinted.

If urine is poured over cold acid, a precipitate may appear which is not albumin. This can happen when the urine is highly charged with urea, in which case crystalline nitrate of urea will separate out, or where urates are abundantly present, in which case the ring will consist of very fine crystals of uric acid, or acid urates. Both of these precipitates are dissipated by heat, and if the nitric acid is previously warmed, they cannot appear. It is better to make the test as just suggested than to use cold acid, and then try to warm a ring formed, as this would cause an admixture of the liquids sufficient to obscure a slight amount of albumin.

It is sometimes recommended to pour the urine in a test-tube, and by means of a pipette, or dropping-tube, allow the acid to flow under it. This is an excellent method of performing the test, but the acid should be slightly warm as before. If only a trace of albumin is present the ring will not appear immediately, but only after standing. It is well, therefore, in doubtful cases, to set the tube aside for twelve hours and then observe it. If a ring is now found it should be very gently and carefully warmed to determine its behavior toward heat, because on standing in the cold a ring of urates might appear.

When this test is applied to old, cloudy, or alkaline urine it should be preceded by this preliminary preparation:

Boil the urine with half its volume of 10 per cent. potassium hydroxide solution and filter. This will usually give a bright, clear liquid, but if not, add two drops of the "magnesia mixture" employed in qualitative analysis and described in the appendix, boil and filter again. The filtrate is now suitable for testing.

The action of the reagents is this: The strong alkali forms a bulky precipitate of the earthy phosphates present which usually settles and leaves the supernatant liquid clear. The amount of alkali taken is sufficient to prevent the coagulation and precipitation of the albumin on boiling, while it serves also to expel ammonia which may be present. If the first filtrate is not perfectly clear the addition of the magnesia mixture accomplishes this by making a new precipitate of phosphates in traces which now leaves it bright.

With the clear filtrate the tests by addition of nitric acid may now be carried out. It must be remembered, however, that as the urine is now strongly alkaline, a relatively large volume of the strong nitric acid must be employed.

The text-books abound in minute descriptions concerning the best methods of conducting this comparatively simple test. The few sources of error which may mislead will now be pointed out. It is, of course, understood that these appear only in the search for small amounts of albumin; that is, for amounts less than one-tenth of 1 per cent. by weight. For greater

quantities the reactions, even when not conducted with extreme care, are usually sharp.

When urine is poured over nitric acid or when the acid is introduced under the urine a *layer of some kind* always appears at the junction of the two liquids. The problem is to decide what this is. The peculiar appearance of a relatively large amount of coagulated albumin is so characteristic that any one who has ever seen it will recognize it again. But a faint cloud or haziness is, at the start, somewhat confusing. A colored layer or ring, which is very common, must not be mistaken for a precipitate or cloud. The normal urine coloring-matters may produce a highly colored ring, and the bands with biliary colors are even deeper. But these color bands or zones are transparent which can be determined by holding the test-tube in the proper light.

Urine very highly charged with urea may give a crystalline precipitate of urea nitrate. This is a very unusual reaction, and the precipitate may be very easily recognized through the form and size of the crystals, which are large flat plates readily seen by the naked eye or by a common magnifying lens. If a urine suspected to contain such an excess of urea be diluted with an equal volume of water before testing the crystals will not appear. Besides, they do not appear when the liquids are warm. The finely granular precipitate of acid urates or hydrated uric acid appears only in a cold liquid, therefore cannot be present to mislead if the test is conducted as directed.

If the special tests indicate the presence of unusually

large quantities of urates the urine may be diluted with an equal volume of water before adding the nitric acid. It occasionally happens that a yellowish white cloud or band appears in this test which is not due to albumin or uric acid. Such a cloud may be caused by the presence in the urine of bodies taken into the system as remedies and which are excreted in but slightly changed form. Derivatives of turpentine and certain resinous bodies are specially liable to behave in this manner. After the use of copaiba balsam, nitric acid throws out from the urine insoluble resin acids which are not dissipated by heat. The precipitate formed by such acids dissolves easily in alcohol and can thus be readily distinguished from albumin which is not soluble. It must be remembered that while pure albumin precipitated by nitric acid is white, that thrown down from urine may be more or less colored from the presence of normal or abnormal coloring-matters.

Attention must be called to a method of conducting the nitric acid test which is frequently employed, but which for small quantities of albumin is very untrustworthy. This method consists in mixing about equal volumes of strong acid and urine, and boiling. This is open to the grave objection that by it the albumin sought may be decomposed and so lost from view. Nitric acid is a very strong oxidizing agent, and albumin a substance easily decomposed. Traces may therefore be lost even by a very short boiling, as may be readily determined by the student by a few experiments with weak albumin solutions.

**Tanret's Mercuric-Potassium Iodide Test.**—A solution of this compound precipitates albumin from acidified urine and is on the whole an extremely delicate reagent. Among the general albumin tests employed by chemists for the recognition of proteids it is always shown that many of these bodies are thrown out from their solutions in the form of complex basic compounds by addition of salts of certain heavy metals. Soluble salts of mercury, lead, and copper give characteristic reactions.

Tanret prepared a well-known and popular test solution in the following manner:

Dissolve 33.12 grams of pure potassium iodide in about 200 cc. of distilled water. Add 13.54 grams of powdered mercuric chloride and warm until, with sufficient stirring, the red precipitate of mercuric iodide disappears, leaving a clear slightly yellowish solution. Dilute this with distilled water to about 800 cc., and add 100 cc. of pure, strong acetic acid. Allow to stand over night if not absolutely clear, and decant from any small precipitate which may have settled out. Dilute then to one liter with distilled water. This solution contains the two salts in the proportion of 4KI to $HgCl_2$.

The test with the reagent so prepared is carried out as follows:

Filter the urine to make it perfectly clear, and add enough acetic acid to give it a good acid reaction. To about 10 or 15 cc. in a test-tube add a very little of the reagent, a drop at a time, from a pipette or dropping-tube. In all not more than five drops should be

added, as this is sufficient to give a strong precipitate if albumin is present. The precipitate is flocculent, and appears as a white cloud or streak, as the first drop of the heavy mercuric solution settles and mixes with the urine. As each following drop mingles with the urine the hazy cloud grows to a precipitate in case the urine contains more than a mere trace of albumin.

The delicacy of the reaction is remarkable. It is said that by it one part of albumin in one hundred thousand parts of urine may be detected. This, however, is probably excessive. One part in twenty-five thousand in a series of tests is nearer the average result. It has been claimed that where the solution is to be kept a long time it is best prepared without the addition of the acetic acid, as this is liable to produce slight decomposition in time. It is likely that the danger of this has been overestimated.

In any event, unless the urine is fresh and slightly acid the addition to it of acetic acid should not be neglected. The use of the acid is said by some writers to be unnecessary, but it has the advantage of disclosing the presence of any quantity of mucin which might interfere with the test. If the acid throws out a cloud of mucin it should be filtered off and then the reagent added.

While this is an exceedingly valuable test certain precautions must be observed in its use. The mercuric solution is similar to one used as a test for alkaloids, and in fact precipitates many of these bodies. Quinine and other alkaloids given as remedies and excreted by the urine would therefore be shown by the test.

Alcohol dissolves these precipitates, however, but is without solvent action on that formed by albumin. Uric acid and urates give precipitates with the reagent if present in large amount. These precipitates can be avoided by diluting the urine before testing, or if formed can be dissipated by slight heat.

Mistaking mucin for albumin can be avoided as shown above. Small amounts of peptones are precipitated by the reagent, but the coagulum disappears by application of heat.

**The Ferrocyanide Test.**—A reagent of great delicacy is potassium ferrocyanide in presence of acetic acid. It shows not only serum albumin, but globulin and perhaps other proteids. It does not give a reaction with peptones.

The test is applied in this manner. The urine must be made as clear and bright as possible by filtration and then strongly acidulated with acetic acid. If a precipitate or cloudiness from mucin appears now, filter again and to the filtrate add four or five drops of a *fresh*, *clear* solution of the ferrocyanide. With even traces of albumin this gives a flocculent yellowish white precipitate.

One of the advantages claimed for this test is that it gives no reaction with the vegetable alkaloids and therefore can be used as a check upon some of the others, the mercuric-potassium iodide test for instance. The precipitate formed, although flocculent, is very fine and can be observed therefore only in clear solution.

A modified form of the test is the following: Mix five drops of the ferrocyanide solution with 5 cc. of 30 per cent. acetic acid. Pour this carefully over an equal volume of clear urine in a test-tube and allow to stand a short time. A white zone at the junction of the liquids shows the albumin.

**The Picric Acid Test.**—Picric acid solutions, pure, or combined with citric, acetic, or other acids, have long been used as reagents for the detection of traces of albumin in urine. In its simplest form the test liquid employed is a saturated aqueous solution of pure picric acid. It gives a very characteristic yellowish flocculent precipitate with even traces of albumin. Another solution frequently employed contains in one liter 10 grams of picric acid and 20 grams of citric acid. It must be made clear by filtration, if necessary, and is applied to clear urine in small quantity by means of a pipette, so as to show a cloudiness as the liquids mingle. The reagent is added gradually to the urine and in all not more than one-half the volume of the latter for a qualitative test.

The real practical value of this test is, in some quarters, still in dispute. It is certainly very delicate, but as it gives precipitates with peptones, alkaloids, urates, mucin, creatinin, and perhaps other bodies, the first result observed is subject to revision. The precipitates formed with these substances and picric acid are dissipated by heat, but there is risk of getting the temperature too high, in which case other pecipitates are liable to be formed, especially with the plain solu-

tion without the citric acid. A urine which yields a precipitate of earthy phosphates by warming will give it at the same temperature in the presence of picric acid. It seems to be true, however, that with citric acid added the interference from phosphates is eliminated, and there remains only mucin as a disturbing element. The danger here is not great, and it is likely that in all cases it can be avoided by adding the citric acid first, filtering if necessary, and then adding the picric acid.

**Trichloracetic Acid Test.**—A saturated aqueous solution of this acid is a very delicate reagent for albumin. The test is made by pouring the solution on the urine in a test-tube. In presence of albumin a white cloud or zone appears at the junction of the two liquids. Other proteids besides serum albumin are coagulated by this reagent, but it appears to be without action on peptones or mucin, and on this account has come into favor among medical men. If much uric acid or an alkaloid is present in the urine, it is best to warm the latter before applying the reagent.

**Other Tests.**—A number of other tests are in use which show very minute traces of albumin, but they seem to possess no advantages over those enumerated above. One of these depends on the precipitation of albumin by phenol and acetic acid; in another picric and hydrochloric acids are used; in a third a strong solution of common salt and hydrochloric acid; and so on, but practically no one will find it necessary to go beyond the six tests given. Indeed, two are by most

authorities generally thought sufficient; *viz.*, the heat test and the nitric acid test. What cannot be shown by these reactions is so minute that for practical purposes it can be neglected generally.

## The Amount of Albumin

It is not alone sufficient that we are able to detect the presence of albumin in urine; we often need to know its amount to determine the practical value of a line of treatment pursued from day to day. To be of the greatest possible service, a method must be so easy of execution that approximately correct results may be obtained by it by the use of simple apparatus and in a short time. Several methods are known by which the amount of albumin in urine can be found. One of these, and the best, may be called the gravimetric method, as by it the albumin is precipitated, collected, and weighed. In another, the albumin is precipitated and its volume measured, while in a third process the amount of albumin is estimated from the degree of turbidity caused by its precipitation in the urine.

Only the first and second methods will be described. The first is employed in exact investigations, and the second in clinical estimations.

**The Gravimetric Method.**—If the qualitative test has shown only a small amount of albumin 100 cc. of the urine should be measured out into a beaker for precipitation. If the qualitative test has given a strong indication 50 or 25 cc. should be taken and diluted to 100. Enough dilute acetic acid is added to the urine to give it a *faint* acid reaction, after which it is brought

up to a temperature of 80° or 90° C. on the water-bath, being stirred, meanwhile, frequently. From time to time the beaker is held up against the light, so that the operator may determine whether the coagulation is complete or not. A satisfactory coagulation is shown by the precipitation of the albumin in large flakes, leaving the surrounding liquid nearly clear. If this is not the case a little more acid should be added, *but very carefully*, and in all but three or four drops, unless the urine was strongly alkaline to begin with.

When the reaction seems to be complete on the water-bath the beaker is placed on gauze and the contents brought to boiling. The precipitate is then allowed to settle. Meanwhile, a small filter of well-washed filter-paper is dried and weighed in a weighing tube. It is plaited and put in a funnel and then the albumin precipitate is collected on it. The precipitate is washed with hot distilled water until it gives no chlorine reaction to the wash-water, then with absolute alcohol, and finally with ether. The funnel with contents is placed in an air-oven and dried at 120° C. The filter is transferred to the weighing tube, and when cold is weighed. The increase in weight gives the albumin. Instead of collecting on paper, a much better plan is to collect on a Gooch funnel of asbestos, when it can be had. This simplifies the test, besides adding to its accuracy. This method consumes a good deal of time, but gives results which are near the truth when it is properly conducted. The best results are obtained when the weight of the precipitate does not exceed 0.3 gram.

**Volume Methods.**—One of the simplest of these is the one proposed by Esbach. In this a special tube is used, called the *Esbach albuminometer*, and a special solution or reagent made by dissolving 10 grams of pure picric acid and 20 grams of pure citric acid in a liter of distilled water. The solution must be filtered if it is not perfectly clear, and is the same as the one used for the qualitative test. The principle involved in the employment of the method is this: The precipitate of albumin and picric acid settles in coherent manner and in a compact volume proportional to its weight, provided certain definite amounts of the reagent and urine are taken. The albuminometer, or measuring tube used, resembles a test-tube of heavy glass about six inches long, and is graduated, empirically, to show how much urine and reagent to take and the amount of albumin obtained expressed in grams per liter, or tenths of 1 per cent. The annexed cut shows the tube and its markings.

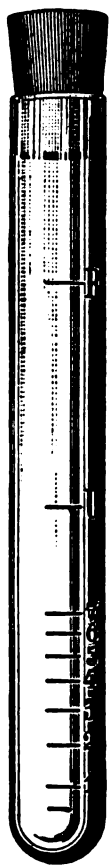

Fig. 2.

The test is carried out in this manner. Urine is poured in, to the mark, and then the reagent, described above, to its proper level. The tube is closed with the thumb and tipped backward and forward eight or ten times until the liquids are thoroughly mixed. It is then closed with a rubber

stopper and allowed to stand in a perpendicular position twenty-four hours. This will give the precipitate time to settle thoroughly after which the amount can be read off on the scale. The results are accurate enough for clinical purposes and by practice can be made to agree moderately well with those found by the gravimetric method.

But to obtain this close agreement a number of precautions must be observed. The volume of the precipitate is in a marked degree variable with the temperature, and with the time given it for subsidence. The empirical graduation is based on the supposition that the test will be made at a temperature of 15° to 20° C., and that the reading will be made at the end of twenty-four hours. If the reading is delayed to two or three days the volume of the precipitate will be found much smaller. At the present time small centrifugal machines are rapidly coming into use to settle urine sediments. Some of these are operated by the Edison electric light current, and give the rotating tubes a high velocity. Where these machines are employed to settle the picric-acid-albumin precipitate, the volume of the latter may be rendered abnormally small and the reading, therefore, prove erroneous.

The volume of the precipitate will depend here, not only on time and temperature, but also on velocity of rotation, and the effect of this factor must be determined for each instrument before it can be accurately used.

In any case in applying this test the urine should not be highly concentrated. The best results are ob-

tained with urine of low specific gravity and with the albumin not over 0.3 of 1 per cent. If a test shows an amount greatly in excess of this the urine should be diluted with a known proportion of water and tested again. On long standing in the cold, a yellowish red precipitate of uric acid sometimes settles out. This need not mislead the analyst, as its color and general appearance are quite distinct from those of albumin.

The precipitates of albumin from urine by whatever means obtained are bulky and lead to the impression that the amount present is much larger than is actually the case. It was at one time customary to speak of 25, 30, and 50 per cent. of albumin, these numbers representing the *apparent* volume of the precipitate in the test-tube. When these light precipitates are collected, properly dried, and weighed, a very different volume is obtained. A urine with 1 per cent. of albumin contains an unusually large amount and in any case seldom more than 10 or 15 grams of albumin occur in the day's urine. Between 1 gram and 5 grams are more common amounts, even in cases of acute albuminuria.

It will not be necessary here to discuss any of the other processes proposed for the rapid determination of albumin in urine. While some of them are certainly much more accurate than the Esbach method as just outlined, they are all too complicated for quick clinical manipulation, and in this respect are no better than the long gravimetric process.

### 2. Serum Globulin

This is an albuminous body resembling the serum albumin in many respects and often, perhaps generally, associated with it.

The globulins, as a class, are distinguished by their insolubility in pure water and very concentrated salt solutions, but they dissolve readily in weak salt solutions. Besides serum globulin there are recognized here *crystallin*, of the crystalline lens, *vitellin*, found in the yolk of the egg, *fibrinogen*, of the blood which gives rise to fibrin on coagulation, *myosin*, found in the muscle substance after death, and finally *globin*, which is a proteid produced by the decomposition of hemoglobin of the blood. The discussion of these bodies belongs in the field of physiological chemistry.

At the present time globulin in the urine has the same clinical significance as serum albumin. Although similar to each other in most points there are several characteristic differences which are taken advantage of as qualitative tests. The general relations of albumin and globulin are pointed out in text-books of chemical physiology where reactions are given for each. But not all of the tests for globulin which we find given in the books are suitable for use in the examination of urine, however, as we are here limited by the presence of other substances. Most of the reactions given above for albumin apply equally well to globulin, and it is only within recent years that attempts have been made to detect one in the presence of the other. Among the methods applicable in the examination of urine the following may be given as most characteristic.

## Qualitative Tests

**Dilution Test.**—Globulin is insoluble in water, but soluble in dilute salt solutions;—hence its solubility in urine. If the latter is diluted until the specific gravity is 1.002 or 1.003 the globulin may separate out. At any rate the addition of a few drops of dilute acetic acid will produce the desired result. A current of carbon dioxide passed into the diluted liquid for several hours accomplishes the same end.

The test may be modified in this manner. Filter the urine if it is not perfectly clear, and then pour it, *drop by drop*, into a tall, narrow beaker of distilled water. If globulin is present it is thrown out as a white cloud which shows as the drops pass down through and mix with the lighter, clear water. The globulin may afterward be confirmed by adding a small amount of salt solution which will cause the precipitate to disappear.

**Sulphate Test.**—Globulin may also be detected by reason of its insolubility in strong salt solutions. To this end treat the urine with enough ammonia water to give an alkaline reaction. This precipitates phosphates and sometimes other salts, which after a time are filtered off, leaving a clear liquid. To this add an equal volume of saturated solution of ammonium sulphate which, in presence of globulin, produces a white flocculent precipitate.

Magnesium sulphate is frequently used for the same purpose, and under some circumstances may possibly be preferable.

In this test there is some danger of confounding albumose with globulin, as the former is also precipitated by ammonium sulphate. But the danger of confusion is small if the conditions given are adhered to; *i. e.*, mix equal volumes of the clear filtered urine and saturated ammonium sulphate solution. For the precipitation of albumose higher concentration is necessary, as will be further explained below.

Where magnesium sulphate is used it is necessary to employ it in very considerable excess. The test is best made by adding the *perfectly pure* salt, in powdered form, to the urine until the latter becomes saturated. Under these conditions globulin, if present, separates. If the sulphate is not pure it may yield a turbid liquid on dissolving, in which the test is uncertain.

The figures given in many of the text-books, as to the amount of serum globulin found in the urine, have in several instances been obtained by methods which are quite erroneous, and are therefore valueless. It appears from the most trustworthy investigations that in all cases of albuminuria globulin is present with the albumin, and in amount varying from 8 to 60 per cent. of the whole proteid content.

### 3. Albumose or Hemialbumose

We have here a representative of an important class of proteid compounds which are derived from the albumins proper. It is well known that in the digestion of native albumins the albumoses appear as one of the stages, and are found, therefore, among the prod-

ucts of peptic and pancreatic action in the body. It is likely that normally albumoses are always converted into peptones before leaving the alimentary tract.

The special significance of the bodies called albumoses in the urine is by no means clear. While certainly a pathological appearance it has not yet been found possible to definitely connect it with any one disease. Its presence has been reported in the urine in several cases of osteomalacia, but it appears by no means to be a constant accompaniment. Observers have called attention to its occurrence during the progress of several other diseases, but without being able to point out any definite relation.

### Qualitative Tests

The recognition of albumose is not a matter of difficulty, as it is distinguished from the other proteid compounds sometimes found in the urine by several well-marked characteristics. It is not coagulated by heat or by the addition of acetic or warm nitric acid, and is very soluble in hot water. It is much less soluble in cold water, but the presence of small amounts of salts seems to increase its solubility here in marked degree.

In presence of albumin or globulin it can be found by the following process unless it is in very small amount. The urine is saturated with pure sodium chloride, which will precipitate albumose if present, and then enough acetic acid is added to give a very strong acid reaction. The mixture is boiled and filtered hot. This treatment throws out both albumin

and globulin, and redissolves a precipitate of albumose which may have formed. The latter would therefore be found in the clear filtrate and sometimes in amount sufficient to precipitate as this cools. The filtrate should therefore be allowed to remain at rest until quite cool. If much albumose is present it will appear as a white cloud. Sometimes, however, it will be necessary to concentrate the filtrate before looking for this reaction, and this is done by evaporating slowly on the water-bath to half the volume. On now cooling, salt will quickly settle out while the albumose precipitates later in flocculent form.

Another test is this: Separate the albumin and globulin by boiling with a small amount of acetic acid without the salt. Filter while warm and concentrate the filtrate to a volume of one-third. Allow to cool thoroughly and add a large excess of *saturated* solution of ammonium sulphate. This gives a white flocculent precipitate of albumose, if present. The precipitate can be collected on a filter and washed with the saturated ammonium sulphate solution and then dissolved in a little distilled water, poured on the filter. This filtrate gives tests with picric acid, potassium ferrocyanide and acetic acid, and other albumin reagents.

If the original urine shows no reactions for albumin or globulin the albumose tests can be applied directly after concentration. The method by precipitation by means of picric acid gives good results. The biuret test is also delicate if the urine is clear and of light color.

The amount of albumose in a urine cannot be found

by any simple method suitable for clinical application. Several processes have been described, but they involve a determination of nitrogen by the Kjeldahl or other method, and can therefore be carried out only in well-equipped chemical laboratories.

### 4. Peptones

As has been explained, peptones are proteid compounds formed from native albumins, globulins, fibrin, etc., in the digestive process. In this respect they are related closely to the albumoses, both differing from the other proteids in important respects; but peptones have further characteristic properties by which they are differentiated in turn from the albumoses.

Peptones may occur in the urine as a result of various abnormal conditions of the body; but their appearance does not depend, like that of albumin, on changes in the circulation or on pathological conditions of the kidney. Their clinical significance, therefore, is very different from that of albumin or globulin.

In cases of phosphorus poisoning the urine has frequently been found to contain peptones, but their presence can usually be connected with the disintegration of pus somewhere in the body. The peptone substances are, therefore, frequently, or perhaps generally, found in the urine in cases of purulent meningitis, purulent pleurisy, in the termination of pneumonia by resolution, and in general under circumstances in which products of suppuration can find their way into the systemic circulation to be eliminated afterward by the kidneys.

Peptones have been reported in erysipelas, pulmonary tuberculosis, acute articular rheumatism, carcinoma of the gastro-intestinal canal, catarrhal jaundice, and in numerous other disorders. The condition in which the urine contains peptones as a result of the breaking up of purulent products is spoken of as *pyogenic peptonuria*, in contradistinction to that in which there is no indication of the existence of a suppurative process. It is claimed that in certain cancerous conditions of the stomach and intestine, the peptones of digestion may find their way into the circulation.

Normally, peptones do not exist in the blood in more than traces, and during absorption from the healthy surfaces of the alimentary tract a change into albumin seems to take place. It is held by some writers that if taken up from an unhealthy surface (ulcerated) this conversion may not take place and peptone unchanged enters the circulation to disappear finally by way of the kidneys and through other channels.

It has been shown also that peptones are a normal constituent of the urine of women in the puerperal state and their occurrence has been pointed out under still other conditions not connected with a suppurative process. However, in the great majority of cases in which their existence is shown the connection with the latter is clear, and the detection of these substances becomes important as an aid to diagnosis.

In regard to the amount of peptone which may be found in the urine we have no very full data. About 5 grams daily appears to be the maximum reported in

cases of croupous pneumonia, but ordinarily the quantity remains far below this.

Many reactions characteristic of peptones are given in the books, but not all of these may be applied to the urine. It should be further said that some of the proteid products described under the name peptone have probably no connection with that found in urine, and such bodies are not necessarily included in the tests given below. Among the characteristics of peptones in general the following may be recalled: 1. They are extremely soluble in water and from such a solution they are not precipitated by boiling or by addition of acid. 2. They are insoluble in alcohol and may be precipitated by an excess from aqueous solution. This precipitate dissolves readily on the addition of plenty of water and is not a permanent coagulum. 3. Peptones are precipitated by acid solutions of phosphomolybdic acid, phosphotungstic acid, and by solutions of several heavy metallic salts. 4. The biuret test is given by peptones. In this test the liquid to be examined is made strongly alkaline with a solution of potassium hydroxide and then a few drops of copper sulphate solution are added. In presence of peptone the light rose-red or violet color is produced. Other forms of albumin behave in the same manner.

Below a few tests which may be applied to urine are given.

**Biuret Test.**—The direct treatment of the urine with strong alkali and the copper sulphate solution is seldom sufficient, it being usually necessary to subject

it to a preliminary treatment to remove substances which interfere with the reaction. A large amount of peptone substance unmixed with albumin or globulin may give a very characteristic color, but in a highly colored urine this may be unsatisfactory, and it is therefore safest to apply first a purifying process. The nature of the preliminary treatment will depend on the presence or absence of albumin or globulin. First, supposing these bodies absent we may proceed as in the following:

**Hofmeister's Tests.**—In order to prove the presence of peptone in urine at least half a liter must be taken and treated with neutral lead acetate in amount sufficient to produce a heavy flocculent precipitate, which is separated by filtration. An excess of the lead must not be used, but the solution is added carefully, a few drops at a time, giving the liquid meanwhile opportunity to settle so that a fresh precipitate can be seen after the addition of more of the reagent. By using proper care the right point can be found when addition of the acetate must cease.

Supposing now that we have a clear filtrate and that no albumin is present, we next add to the filtrate a little hydrochloric acid, and then a solution of phosphotungstic acid in hydrochloric acid as long as a precipitate forms. If peptone is present it is contained in this precipitate which must be separated immediately by filtration, and washed on the filter with dilute sulphuric acid (5 per cent.) until this passes through without color. The moist precipitate is then

transferred to a small dish and mixed with a slight excess of barium carbonate or with enough crystalline barium hydroxide to give a slight alkaline reaction after thorough stirring. A little water is added and the whole is heated on the water-bath about ten minutes and filtered. The filtrate is then tested for peptone by the biuret test, or by the picric acid solution, as used in other proteid tests.

If albumin is present in the original urine it cannot be completely removed by lead acetate as just explained. The reaction with acetic acid and potassium ferrocyanide will usually show it in the filtrate from the lead and it must be removed as follows, this being accomplished by precipitating it with ferric oxide. Add to the 500 cc. of filtrate a small amount of sodium acetate solution and then some ferric chloride, after which the urine is made neutral with sodium hydroxide and boiled. The iron should be completely precipitated, carrying with it the albumin. The solution is filtered, allowed to cool, and tested for albumin. If free from this it is ready for the peptone test, beginning with the addition of hydrochloric acid. If albumin is still present, add a little more iron or alkali, as experiment will decide, and boil again.

The phosphotungstic acid solution is made up by various formulas, but as a reagent for urine the following method is recommended: A solution of pure sodium tungstate is made of about twenty per cent. strength. To this is added either glacial phosphoric acid or sirupy phosphoric acid and the mixture boiled, the acid being added in sufficient amount to give a

strong acid reaction. With glacial phosphoric acid the proportion should be about one part to four of the tungstate. The boiled mixture is allowed to cool thoroughly, and to it is added about one-fifth its volume of strong hydrochloric acid. Allow the mixture to stand and pour off the clear liquid from any precipitate which may settle out. The complex phosphotungstic acid is one of the few reagents which completely precipitate peptones and other albuminous bodies. In this case it is applied after the other albumins are thrown out by other means and serves to take the peptone away from coloring and equally objectionable substances.

**Negative Tests.**—Peptones are not precipitated by heat or by the addition of hydrochloric, sulphuric, nitric, or acetic acid. The reaction with potassium ferrocyanide and acetic acid, characteristic for the other albumins, is not given by the peptones.

They differ from the albumoses in not being thrown down by excess of ammonium sulphate, from which it follows that the test below can sometimes be applied for the detection of peptones in presence of albumin or albumose.

Precipitate 50 to 100 cc. of urine, acidulated with a few drops of acetic acid, by boiling. Filter, concentrate the filtrate to a volume of 5 cc., allow to cool and add 50 cc. of cold saturated solution of ammonium sulphate and then some of the pure crystals so that the whole liquid is completely saturated. Then filter and to the filtrate apply the biuret test with a large

excess of alkali and a trace only of copper sulphate, or apply the phosphotungstic acid test.

If the latter test is used the filtrate from the albumose should be diluted with two volumes of distilled water. The solution of phosphotungstic acid gives a precipitate with normal urine, and also with the undiluted ammonium sulphate filtrate.

But reduced with water as directed no precipitate of the ordinary urinary constituents appears. A slight opalescence only may result, while in presence of even traces of peptones there is a marked precipitate. The reaction between phosphotungstic acid and peptone solutions is one of extraordinary delicacy, so that traces show even after the above treatment.

In making the biuret test in a solution containing a large amount of ammonium sulphate some color is always obtained. In absence of peptone it is the characteristic blue of the copper sulphate and ammonia reaction, but in presence of peptone the shade is reddish violet.

### 5. Mucin

In small amount mucin is probably present in all normal urines, in the case of women coming especially from the vagina. In moderate amount it has, therefore, no pathological significance. If coming from the urinary tract in more than minute traces it usually indicates an irritated condition or catarrh of the passages and then has clinical interest.

Urine containing mucin in large amount is turbid when passed; with a smaller amount it may be clear at first but on standing deposits a cloud which settles

nearly to the bottom of the vessel and there floats in loose form, instead of compact as with other sediments. This cloud does not clear up by the addition of acetic acid or dilute nitric acid.

In clear urine containing mucin a flocculent, hazy precipitate is formed by the addition of acid. The test is best made by pouring some acetic acid into a test-tube and then carefully an equal volume of urine so as to mix the two liquids as little as possible. A mucin cloud appears in the urine layer above the zone of contact of the liquids. An albumin cloud makes its appearance lower, or at the zone. In testing for mucin in presence of albumin the main portion of the latter should be precipitated first by boiling and filtering. The mucin test can be applied to the cold filtrate by addition of acetic acid.

Mucin as well as albumin is precipitated from urine by the addition of an excess of strong alcohol (three volumes to one). After some hours the precipitate may be collected on a filter and washed with alcohol. It is then washed with warm water which dissolves the mucin. This may be recognized in the aqueous solution by the addition of acetic acid.

Small amounts of mucin are so frequently mistaken for traces of albumin that attention must be paid to the methods of distinguishing between them. From what has been said it will be understood that the cloud which appears as a diffused haze in testing for albumin by an excess of acetic acid or a very small trace of nitric acid may be due to mucin and not to albumin as frequently assumed by mistake. A proper excess of nitric acid redissolves mucin, but not albumin in the cold.

# CHAPTER III

## THE TESTS FOR SUGAR, ACETONE, ACETOACETIC ACID, AND OXYBUTYRIC ACID

On the question of the occurrence of sugar in the urine a vast amount has been written. At one time, indeed until within quite recent years, it was generally assumed that normal urine contains no sugar or carbohydrate of any kind. But present methods of research seem to throw doubt on the truth of this view. It is not possible to separate small traces of sugar from a complex liquid like the urine so that the body separated may be recognized by its sensible properties. On the contrary we must depend on the results of certain reactions given by sugar solutions and in many instances by other organic bodies, and it is on the proper interpretation of these reactions that the authorities differ. Some of these reactions for traces will be explained below. In this place it suffices to say that the leading physiological chemists of the present time are nearly unanimous in holding that traces of the sugar known as dextrose exist normally in urine, in other words, that there may be such a condition as *physiological glycosuria* as distinguished from the well-known pathological condition characterized by the presence of relatively large amounts of sugar in the urine and named *diabetes mellitus.*

The amount of sugar believed to be normally present is very small and cannot be recognized by the first three or four tests given below. An amount of sugar in the urine sufficient to have clinical importance is readily recognized by many tests.

The characteristics of urine in true diabetes are these : It has a specific gravity higher than normal, usually between 1.030 and 1.040, and *this with a greatly increased quantity.* A high specific gravity with small volume, it has been shown, need have no special clinical importance as such a condition can result from many causes outside of disease. Diabetic urine is usually light in color and prone to speedy decomposition by fermentation. The amount of sugar which can be present in advanced stages of *diabetes mellitus* may be very large. It is said that as much as 1,000 grams of dextrose has been passed with the urine in one day in extreme cases ; but the amount usually coming under the observation of the practitioner is far below this, 10 to 100 grams being much more common amounts. In typical diabetes the *percentage* amount of the normal urine constituents is usually greatly diminished because of the great dilution with water, but the *actual* amount excreted in twenty-four hours may be increased.

It is well known that sugar may temporarily occur in the urine from a variety of causes. It has been found after the absorption of several poisons, and in cases of carbon monoxide poisoning; also in the course of certain diseases.

The amounts present in these circumstances are

usually small, and disappear with other symptoms of the disorder. The *continued* presence of considerable quantities of sugar is characteristic of only one disease; *i. e.*, the *diabetes mellitus*. This fact should be borne in mind in the practical examination of urine and tests should be repeated from time to time, unless the other clinical evidence is sufficient to immediately confirm the indication of the chemical test.

## Qualitative Tests

The tests for sugar in urine depend on several distinct general reactions. The most common of these reactions is that due to the oxygen absorbing power of alkaline dextrose solutions. The absorption of oxygen may give rise to a solution of characteristic color and odor as in the first one of the tests given below, or to certain precipitates formed by the abstraction of oxygen from metallic combinations in the test solutions employed.

This oxygen absorbing or reducing power of sugars in general is due to their peculiar chemical structure, inasmuch as they must be regarded as the aldehyde or ketone derivatives of polyhydric alcohols. Common dextrose is probably an aldehyde, and therefore active in its reducing power. The sugar tests which are most commonly employed in urine analysis will be explained at some length because of their great importance.

**Moore's Test.**—This depends on the reaction between grape sugar and strong alkali solutions. When a solution of sugar or diabetic urine is mixed, without

heating, with a solution of sodium or potassium hydroxide, no change is at first apparent unless the amount of sugar present is large or the alkali very strong. But on application of heat, even with weak sugar solutions, a yellow color soon appears which grows darker, becoming yellowish brown, brown, and finally almost black, while an odor of caramel is quite apparent. The strong alkali-sugar solution absorbs atmospheric oxygen, giving rise to a number of products among which lactic acid, formic acid, pyrocatechin and others have been recognized. The brown color is due to other unknown decomposition products.

This is a good reaction for all but traces of sugar, as the intense dark brown color and strong odor are not given by other substances liable to be present in urine.

But traces of sugar cannot be recognized by this test with certainty, as the color of normal urine even is darkened to some extent by the action of alkalies.

Urine containing much mucin becomes perceptibly darker when heated with sodium, potassium, or calcium hydroxide solutions.

**The Trommer Test.**—This is one of the oldest and best known of the tests for the recognition of sugar in urine, and has been referred to before. It is performed by adding to the urine an equal volume of 10 per cent. solution of sodium or potassium hydroxide and then a *very few drops* (three or four to begin with) of dilute solution of copper sulphate.

Solutions of alkali and copper sulphate alone give

a blue *precipitate* of copper hydroxide, but in presence of sugar and certain other bodies a *deep blue solution*, and not a precipitate, is formed. Therefore, if the urine tested contains sugar the first indication is a more or less blue solution, stable for some time in the cold. On standing, however, the liquid turns greenish, and finally deposits a yellow precipitate. This change takes place immediately on application of heat, the greenish-colored precipitate turning yellow, and finally red by boiling. Copper suboxide precipitates, and this is the second and characteristic stage of the Trommer reaction.

Several substances can give the first stage, but dextrose is the only body liable to be present in the urine which can give a good indication in the second.

The test must, however, be used with certain precautions. Albumin, if present, must be coagulated and filtered off. The amount of copper sulphate used must be small, because if only a trace of sugar is present and much copper is used the latter will give a blue precipitate which does not redissolve, and which turns black on boiling, thus obscuring a sugar reaction which may be given at the same time.

In adding the copper sulphate it is best to pour into the test-tube containing the urine and alkali, first about three drops of a five per cent. solution. If this appears to give a yellow color on boiling, which does not turn black, more should be added, and this continued until a yellow or red precipitate is formed. A black precipitate on boiling shows that too much copper has been added, and that probably sugar is absent.

The active body in producing the reaction is copper hydroxide, but this must be in solution to act as a good oxidizing agent with sugar; and the test, therefore, becomes uncertain or unsatisfactory if so much copper is added that the hydroxide formed cannot be dissolved by the sugar which may be present. In doubtful cases it becomes necessary to make several trials before the right proportion between urine, alkali, and copper solution is found. In the solution, on completion of the reaction, several oxidation products of sugar are found, among which are formic acid, oxalic acid, tartronic acid, etc. But the complete reaction is obscure. In order to avoid the indicated uncertainty of the Trommer test when used for small amounts of sugar the next one was proposed.

**The Fehling Solution Test.**—Fehling suggested the use of a solution containing along with the copper sulphate and alkali a tartrate to dissolve the copper hydroxide formed by the first two. Many substances besides sugars, referred to in the last paragraph, have the power of dissolving copper hydroxide with a deep blue color. Among these may be mentioned tartaric acid and the tartrates, glycerol, mannitol and others of less value.

A solution prepared by mixing certain quantities of alkali, copper sulphate, and either one of these bodies, with water in definite proportions remains perfectly clear when boiled. But if a trace of dextrose (or several other sugars) is present the usual yellow precipitate forms.

In the preparation of the original Fehling solution

it was assumed that, in the presence of alkali, the reaction takes place between exactly five molecules of copper sulphate and one of dextrose, and on this assumption the solution was made of such a strength that 1 cc. would oxidize five milligrams of dextrose. We have, therefore, the relation:

$$5(CuSO_4 \cdot 5H_2O) : C_6H_{12}O_6$$
$$1248 : 180 :: 34.66 : 5.$$

Each cubic centimeter of the prepared Fehling solution must contain 34.66 milligrams of pure crystallized copper sulphate and just how to best combine this with the necessary alkali and tartrate is shown in the appendix.

The great advantage which this solution has over the Trommer test is found in the fact that it may always be used safely in excess. With only a trace of sugar there is no danger that the copper will precipitate as black hydrated oxide. In performing the test a few cubic centimeters of the Fehling solution (4 or 5) are poured into a test-tube, diluted with an equal volume of water, and boiled. The solution must remain clear. Then the urine is poured in, at first about half a cubic centimeter, and the mixture boiled. If sugar is present in amount above one-tenth of 1 per cent. it should show with the volume of urine taken. For smaller amounts of sugar more urine must be added, and the mixture boiled again.

When normal urine is heated with Fehling solution, a greenish flocculent precipitate usually makes its appearance. This has no significance as it is due to the

phosphates normally present which come down when the reaction is made alkaline. Many urines produce a clear dark green solution when heated with the Fehling solution. This is a partial reduction reaction and like the other has no special importance as urines free from sugar give it. At other times urines free from sugar yield an almost colorless mixture when boiled with the Fehling solution. These peculiar reduction effects are due to the presence of uric acid, creatin, creatinin, pyrocatechin and several other substances and are generally characterized by discharge of the deep blue color of the solution without precipitation of the copper suboxide. Certain substances taken as remedies give rise to products in the urine which exert a similar action. Occasionally, however, the amount of uric acid is so large that the reduction is accompanied by actual precipitation of the copper as red oxide. This fact is of interest as it makes the test, at times, somewhat uncertain, but it is a very simple matter to determine whether or not a great excess of uric acid is present, as will be pointed out later. The liability to error in the Trommer test from these causes is less than in the Fehling test, but notwithstanding this the latter must still be regarded as the better test practically, because of its great convenience and the sharpness of the reaction with even traces of sugar. The ingredients of the Fehling test are best kept in separate bottles closed with rubber stoppers. A very convenient arrangement is explained in the following paragraph.

Two bottles, each holding about 200 cc., are fitted

with perforated rubber stoppers. Through the opening in each stopper the stem of a 2 cc. pipette with very short tip is passed, and left in such a position that when the bottles are half filled the bulbs and stems to the mark will be covered with the liquid. One bottle contains the standard copper sulphate solution, the other the mixture of alkali and tartrate solution. The rubber stoppers should be covered with vaseline so that they will permit the pipette stems to slide easily in the perforations, and also close the bottles perfectly. When the stoppers are inserted the pipettes should stand full to the mark, ready for use.

On withdrawing the stoppers with forefinger closing the pipettes, exactly 2 cc. of each liquid can be taken out without delay, and on mixing in a test-tube yield the Fehling solution, fresh and ready for use, directly, or after dilution with distilled water, as thought necessary. As the solutions are used the pipette stems are pushed farther through the stoppers so as to leave the marks always at the surface of the liquids. The solutions may be kept in this manner for years, and their use is not attended with any inconvenience. The open ends of the pipette stems should be kept closed with small rubber caps, or a bit of soft paraffin wax. The mixed Fehling liquid does not keep well unless prepared with certain unusual precautions, and therefore several other single solutions have been suggested, as described in the next paragraph.

**Other Copper Solutions.**—The original Fehling solution has been modified in various ways. Most of these

modifications consist in mere changes in the proportions of the ingredients dissolved. Two, however, may be considered as fundamentally different.

Loewe (1870) recommended a solution made by dissolving copper sulphate in water, adding solution of sodium hydroxide and then glycerol. For certain purposes copper hydroxide was found to possess advantages over the sulphate. The preparation of the Loewe solutions is described in the appendix. The claim was made by Loewe that the addition of glycerol prevents the spontaneous decomposition of the blue solution, which may, therefore, be kept mixed. While this is not absolutely correct it is true that the glycerol solutions keep much better than the mixed tartrate-alkali copper solutions as usually made, and have, therefore, found favor with some physicians.

Schmiedeberg (1886) described a solution containing in one liter 34.63 grams of crystallized copper sulphate, 16 grams of mannitol and 480 cc. of sodium hydroxide solution of 1.145 sp. gr. This solution is easily prepared and has also the advantage of permanence.

Both the Loewe and the Schmiedeberg solutions have suffered slight alterations, without, however, being improved.

The second suggestion of Loewe, *i. e.*, to use copper hydroxide instead of sulphate, has not been generally followed, but it certainly has in some cases decided advantages. The final reaction in all these tests is the same as with the Trommer or Fehling test.

**The Bismuth Test.**—Böttger found (1856) that in presence of alkali, bismuth subnitrate is reduced to the metallic condition by the action of dextrose in hot solution. As a urine test he recommended to make it strongly alkaline with sodium carbonate, and then add a very small amount, what can be held on the point of a penknife, of the pure bismuth subnitrate. On boiling the mixture the insoluble bismuth compound, which settles to the bottom, turns dark if sugar is present.

The test is at present carried out by adding to the urine in a test-tube an equal volume of 10 per cent. solution of sodium or potassium hydroxide, and then the subnitrate. Boiling gives the reaction as before. In absence of sugar (or albumin) the bismuth compound remains white.

In performing this test only a very small amount of the subnitrate should be taken. This is absolutely necessary in the detection of traces of sugar. In this case the reduction is but slight, and not much black powder of bismuth or its oxide can be formed. If a great excess of the white subnitrate is taken it may be sufficient to completely obscure the reduction product. It is frequently well to use not more than four or five milligrams of the subnitrate.

The black precipitate formed was at one time supposed to be finely divided metallic bismuth. Later investigations seem to show that it consists essentially of lower oxides of bismuth. This test has certain advantages over the copper tests. It is easily made, and with materials everywhere obtainable in condition of

sufficient purity. Furthermore, the reaction is not given with uric acid, which it will be remembered may act on the Fehling solution if excessive.

Albumin, however, interferes with the test, as it gives, also, a black precipitate when boiled with alkali and the bismuth subnitrate. In this case the albumin gives up sulphur and forms bismuth sulphide.

If albumin is present in a urine it should be coagulated and filtered out before trying the bismuth test. Bruecke recommends to coagulate by means of a solution of potassium bismuth iodide, the excess of bismuth serving to complete the sugar test. The reagent for this purpose is made by dissolving freshly precipitated bismuth subnitrate in a hot solution of potassium iodide by the aid of some hydrochloric acid. This is the solution previously recommended by Fron for the precipitation of alkaloids, and is made by dissolving 7 grams of potassium iodide in 20 cc. of water to which after heating 1.5 grams of the bismuth subnitrate and 1 cc. of pure strong hydrochloric acid are added. The mixture must be kept hot until all is dissolved, resulting in an orange-red solution.

This reagent precipitates albumin, but as it is rendered turbid by water the amount of acid necessary to prevent this for a given volume must be ascertained before it can be used with urine. This can be determined by adding a little of it (a few drops) to some water in a test-tube, and then dilute hydrochloric acid until the precipitate just disappears. The test proper is then made by taking the same quantity of urine and adding the same amount of acid and the reagent.

Albumin and other disturbing substances precipitate, and can be filtered off. The clear filtrate should not be made turbid by acid or the reagent. It is next made strongly alkaline with potassium or sodium hydroxide and then boiled. In presence of sugar a black precipitate is formed as before.

After adding the reagent it is necessary to wait several minutes for a possible precipitate to form and settle. The addition of alkali to the filtrate produces a bulky white precipitate of bismuth hydroxide which is readily reduced at the boiling temperature by sugar present. If only traces of sugar are present the boiling must be long-continued to obtain the black precipitate. What was said above about the danger of obscuring this precipitate by the white bismuth compounds obtains also here.

When only a small amount of sugar is suspected it is best to allow the bismuth hydroxide precipitate to partially settle, and then to pour off the supernatant alkaline urine with a little of it. In this manner the amount of the bismuth compound which finally enters into the test is so small that it should all be reduced by even a trace of sugar, on subsequent boiling. When carefully performed this modification of the original Böttger test is a practically good one. It is not as sensitive as the Fehling test but shows traces of sugar of clinical importance.

**Fallacies in the Reduction Tests.**—It has been shown above that several bodies normally found in urine are able to reduce the alkaline copper solutions. Some

of these interfere with the bismuth reactions also, but not to the same degree ; but attention must be called to another source of error which is very important. In warm weather it is often desirable to add something to urine to prevent its rapid decomposition, and several substances have been suggested for the purpose. The best known are chloral, chloroform, salicylic acid, phenol, and formaldehyde. Unfortunately all of these except phenol have rather a marked action on the copper solutions. As a preservative phenol is objectionable from other standpoints. Urine intended for sugar tests should be tested as soon as possible after collection, and no foreign substance should be added as a preservative. Neglect of this very simple and obvious precaution has caused many serious blunders, especially in the examination of urine from applicants for life insurance.

**The Phenylhydrazine Test.**—In this test a reaction discovered a few years ago has been applied by v. Jaksch to the examination of urine. Add to about 10 cc. of urine 0.2 gram of phenylhydrazine chloride and a slightly greater amount of sodium acetate. Warm the mixture gently, and if solution does not take place add half the volume of water and heat half an hour on the water-bath. Then cool the test-tube by placing it in cold water and allow it to stand. If sugar is present a yellow precipitate settles out, which consists of minute needles generally arranged in rosettes, visible under the microscope. Albumin does not obscure this test, but if much is present it is best to coagulate

it as well as possible by boiling, and filter. The yellow precipitate is called phenylglucosazone.

For the detection of traces of sugar by this method it is necessary to use more urine and more of the reagents. 50 cc. of urine with 2 grams of phenylhydrazine chloride and 3 grams of the acetate may be taken.

Phenylglucosazone melts at 205° C. and a determination of the melting-point may be made as a confirmatory test. For this purpose the supernatant liquid is poured off and the fine yellow crystals are washed with water by decantation. They are transferred to a small watch-glass, allowed to dry over sulphuric acid in a desiccator, and are then ready for the test. Melting-points are usually found by placing a small amount of the substance in question in a thin narrow tube, which is fastened to a thermometer by means of rubber bands. The substance in the bottom of the tube must be near the bulb of the thermometer. The bulb and bottom of tube are then immersed in a beaker of oil or sulphuric acid which is gradually heated until the substance begins to fuse. The temperature indicated by the thermometer is taken as the melting-point. For best methods of working this test of finding the fusing-point some standard manual of organic chemistry should be consulted.

On the whole, it must be said that this reaction is of very limited applicability in urine analysis. It has value only when the copper or bismuth methods are insufficient to decide concerning the presence or absence of sugar. In cases having real clinical importance such uncertainty is rare.

**The α-naphthol Test.**—This depends on the reaction between α-naphthol and sugar in presence of sulphuric acid, and was discovered by Molisch. Take about a cubic centimeter of urine, previously diluted with five to ten volumes of water, and add to it two drops of a 20 per cent. solution of α-naphthol in alcohol. Then add about half a cubic centimeter of strong sulphuric acid and agitate. A blue color indicates sugar. If the acid is carefully added so as to flow under the lighter liquid, a blue zone is formed between them. By diluting largely with water and shaking, a violet precipitate is produced.

This method is exceedingly delicate, but unfortunately is not characteristic, as many substances show the same result. The trace of sugar, or similar body, normally present, gives a marked reaction; hence the direction to largely dilute the urine before adding the reagent.

The α-naphthol may be replaced in this test by a 20 per cent alcoholic solution of thymol. The mixture becomes dark red and carmine-red on dilution with water. It has been shown that these color changes depend on the formation of small amounts of furfural by action of sulphuric acid on traces of carbohydrates and the subsequent combination of the furfural with the α-naphthol or thymol. However, not only carbohydrates, but also albumins and many other substances yield furfural in this manner and in normal urine some of these substances may be always present.

Molisch claims that the reaction found with highly diluted urine is a sugar reaction, that in condition of

high dilution other bodies which may possibly be present cannot give this test. A color still shows when normal urine diluted 100 times is used, and on this behavior, partly, the claim that sugar is normally always present in urine is made. It will be seen from this that the test is too sensitive for ordinary clinical needs, but as a laboratory test it is valuable. By attentive study of the behavior of diluted normal and diabetic urines the chemist soon learns to recognize the deeper colors obtained with the latter and is therefore able to employ the test in the way of confirmation.

**The Fermentation Test.**—When yeast is added to urine containing sugar and the mixture left in a moderately warm place the usual fermentation soon begins which is shown by two principal changes. Carbon dioxide is given off, which may be collected and identified, and the mixture becomes lighter in specific gravity. When only traces of sugar are present the test by collection and identification of the carbon dioxide frequently fails because of the solubility of the gas in the liquid.

The variation in the specific gravity is an indication of greater value, as it can be readily observed with proper appliances. The test has practical value, however, only as a confirmation of some other one. If by the copper solutions, for instance, a strong indication is obtained which it is suspected may be due to an excess of uric acid, the reaction by fermentation may be resorted to because *only sugar* will respond to it.

The test may be made by pouring 100 cc. of the urine into each of two bottles or flasks. To one, half a cake of compressed yeast, crumbled, is added; the other is left pure. The bottle with the yeast is closed by means of a perforated stopper (to allow escape of gas), while the other is tightly corked. The two are left side by side, in a warm place about twenty-four hours. At the end of this time a test of the specific gravity of the contents of both bottles is made. If sugar is present to the amount of one-half per cent. the specific gravity of the yeast-bottle should be perceptibly lower.

The test is frequently recommended as a quantitative one, as there is a fairly definite relation between amount of sugar and loss in density.

**Other Sugar Reactions.**—Many other tests have been proposed for the detection of sugar in urine. A few of these will be referred to briefly in this place.

One of these, the picric acid test, is based on the fact that a urine containing sugar when mixed with solutions of potassium hydroxide and picric acid and boiled, turns a dark mahogany-red from formation of picramic acid.

When urine is made strongly alkaline with potassium hydroxide and treated with a weak solution of diazobenzene sulphonic acid in water, it turns reddish yellow if sugar is present, and becomes afterward claret-red and finally dark red if much is in solution. The reaction is delicate, but is given by other bodies than sugar.

Another test depends on the reaction between sugar

solutions and indigo-carmine in presence of alkali. The urine is made alkaline with sodium carbonate and treated with indigo-carmine until a deep blue is obtained on heating. If sugar is present, on longer heating the color fades to yellow by reduction. The color returns by cooling and shaking with air.

Weak aqueous solutions of methylene-blue are decolorized when boiled with alkaline dextrose solutions. To apply this in urine analysis a solution of the methylene-blue is made by dissolving about 30 milligrams in 100 cc. of water. In a test-tube take 5 or 6 cc. of this solution, add 2 cc. of a 5 per cent. potassium hydroxide solution and then the diluted urine. On boiling, the color fades or disappears completely if much sugar is present. In urines containing much sugar a reaction is given distinctly with 2 cc. after tenfold dilution.

A very characteristic reaction is given by the aid of the coloring-matter known as safranine. This occurs in commerce as a mixture of three related bodies having the empirical formulas, $C_{21}H_{21}N_4Cl$, $C_{20}H_{19}N_4Cl$, and $C_{19}H_{17}N_4Cl$. It is soluble in water with a red color and a solution of 1 part to 1000 of water is employed as the reagent. A 5 per cent. potassium hydroxide solution is also used and the test is made by adding to a small volume of the suspected urine an equal volume of the alkali solution and then the safranine solution in like amount. About 3 cc. of each should be taken, and the mixture then boiled. In presence of sugar to the extent of one-tenth per cent. or more the mixture is decolorized, a pale yellow result-

ing. With stronger sugar urines more of the safranine solution is decolorized and a rough measure of the amount of sugar present may be made by noting the volume of safranine solution in which the color is destroyed by the original urine taken. If the test-tube in which the test is made be allowed to stand exposed to the air the red color will return soon in the upper part of the liquid by oxidation. The loss of color in the mixture depends on the reduction of the safranine; fortunately but few substances likely to be present in urine show the same behavior, and on the whole the test may be considered the best of the color reactions. It has been very highly recommended and may be used when there is doubt concerning the copper or bismuth indications.

### The Amount of Sugar

It is not always sufficient to be able to detect the presence of sugar in urine. A knowledge of the amount is frequently of the greatest importance. A number of methods have been proposed by which a quantitative determination can be made, some of them crude and of little practical value, while others give, when properly carried out, results which are accurate. The methods in general may be divided into four groups, depending on the

(1) Reduction of solutions of heavy metals, and measurement of the amount of reduction.
(2) Change of color produced in organic solutions, by action of sugar, the depth of final color being proportional to the amount of sugar.

(3) Results of fermentation with measurement of change in specific gravity of the urine or measurement of evolved carbon dioxide.
(4) Observation of rotary polarization of light.

## Methods by Reduction of Metallic Solutions

The reduction methods are illustrated in the use of the Fehling solution as a qualitative test and in the bismuth tests. The general principles involved in making a quantitative determination of sugar by aid of the Fehling solution are the same as those involved in making other volumetric analyses with standard solutions, and are fully explained in the author's work on chemical physiology, or in any one of the standard manuals of volumetric analysis in general use. A measured volume of the properly prepared Fehling solution is poured into a flask and brought to the boiling-point. Then from a burette the dilute sugar solution is run in slowly, a few cubic centimeters at a time, until the deep blue of the copper solution is just discharged, leaving a pale yellow. At this stage the copper is all reduced to the condition of insoluble red suboxide, $Cu_2O$. If the Fehling solution is used in undiluted condition and the sugar is present in solution of about 1 per cent. strength, then each cubic centimeter of the Fehling solution is reduced by 4.75 milligrams of dextrose. If the Fehling solution is diluted with four volumes of water before use its oxidizing power is slightly greater, each cubic centimeter being equivalent to 4.94 milligrams of dextrose; 25 or 50 cc. of the Fehling solution should be taken and, if

undiluted, the 118.75 or 237.5 milligrams of sugar required for reduction must be contained in the volume of solution added from the burette. A simple calculation gives the strength of the saccharine liquid in grams per liter, or in per cent. by weight if the specific gravity is known.

When applied to the urine, however, the process requires certain modifications because of the fact that this secretion contains always a number of substances which interfere to some extent with the normal reduction and precipitation of the copper suboxide. The determination of dextrose in aqueous solution by the Fehling liquid is a problem of extreme simplicity, but in urine the case is somewhat different.

If we measure out 50 cc. of the mixed Fehling solution, heat it to boiling and then run in the saccharine urine from a burette, it frequently happens that a greenish yellow *muddy* precipitate forms which does not turn bright red and which, instead of quickly settling to the bottom of the flask, remains suspended and makes it impossible to observe the disappearance of the blue color indicating the end of the reduction. This difficulty may be largely obviated by working with solutions of greater dilution, as explained in the following paragraph.

**Determination by Fehling Solution.**—Prepare a Fehling solution as shown in the appendix and then accurately mix it with four volumes of distilled water, that is, to 100 cc. add 400 cc. of water, to 50 add 200, or to 25 add 100. In any event the dilution must be accurately made. One cubic centimeter of this liquid

will oxidize almost exactly one milligram of dextrose as shown above, provided the sugar is in approximately 1 per cent. solution. For all practical purposes of urine analysis the oxidizing power may be considered the same in a solution of one-half per cent. strength, and only very slightly increased in still weaker solutions. Therefore, before beginning the test dilute the urine, if necessary, accurately with four or nine volumes of water. This can be done by making 50 cc. up to 250 or to 500 cc. and mixing well by shaking.

With the diluted urine so prepared proceed as follows: Measure out 50 cc. of the dilute Fehling solution, pour it in a flask and heat to boiling on gauze. Fill a burette with the diluted urine and when the solution in the flask is actively boiling run in about 3 cc. Boil two minutes, remove the lamp, and wait half a minute to observe the color. If blue is still visible, heat to boiling again and run in 3 cc. more. After boiling two minutes as before, wait a short time and observe the color near the surface of the liquid in the flask. If still blue repeat these operations until on waiting it is found that the blue has given place to a yellow. The urine should be so dilute that at least 10 cc. must be run in to reduce all the copper hydroxide.

When the volume required is found to within 2 or 3 cc. a second experiment must be made, the urine being added very gradually now, without interrupting the boiling longer than necessary, until the first of the limits between which the correct result must lie, as shown by the former test, is reached. From this point

the addition of the urine is continued, with frequent pauses for observation of color, until the reduction is complete. The volume of urine used contains 50 milligrams of sugar.

If the preliminary experiment shows that the urine is strong in sugar and that the reduction is easy, that is, that the cuprous oxide separates and settles readily, the second test may advantageously be made with 50 cc. of a stronger Fehling solution. With many strong diabetic urines it is possible to use the undiluted copper solution with the oxidizing power of 4.75 milligrams of sugar to each cubic centimeter. The difficulties in this test have been very much overestimated; with a little practice any one can make a good sugar determination in urine. The important point is to find by a few simple preliminary tests the best conditions of dilution of Fehling solution and urine to give a precipitate which settles readily. With this information, and it can be acquired in a few minutes, the actual quantitative experiment can be easily made.

As an illustration of the calculations involved let it be assumed that 50 cc. of the dilute Fehling solution is reduced by 11 cc. of urine. Each cubic centimeter of the urine must therefore contain 4.54 milligrams of sugar. If the urine were undiluted this corresponds to 4.54 grams to the liter. If it had been diluted with 9 volumes of water the result must be multiplied by 10, giving as the original strength 45.4 grams per liter. If the specific gravity of the urine were found to be 1.032, the percentage strength would be

$$\frac{4.54}{1.032} = 4.39.$$

**The Use of Pavy's Solution.**—To avoid some of the difficulties in the titration of diabetic urine by the Fehling solution, Pavy suggested a solution containing ammonia. If a solution of dextrose is run into a boiling copper solution containing ammonia in considerable quantity the copper is gradually reduced, giving finally a clear, colorless solution instead of a red precipitate. The end of the reduction is, therefore, indicated by disappearance of color alone. The preparation of the Pavy solution is given in the appendix. Its strength as there described is just one-tenth of that of the common Fehling liquid; that is, 100 cc. oxidizes 50 milligrams of dextrose. The test is performed in a flask, as is the Fehling titration; but as the solution is easily changed by atmospheric oxidation, just as soon as it begins to hold some reduced copper, precautions should be taken to exclude the air during titration. This can be done by passing a slow current of illuminating gas or hydrogen through the flask during the test, or, better, by adding to the contents of the flask enough white paraffin wax to form, on melting, a protective liquid layer several millimeters in depth.

The titration is carried out as follows: Measure 100 cc. of the ammoniacal copper solution into a flask holding about 300 cc. Throw in some small pieces of pumice stone to prevent "bumping," and then heat to the boiling-point on wire gauze. The sugar solution must be dilute, and should be contained in a burette with a delivery tip bent to one side and then down, so that the contents of the burette can be added

slowly but continuously to the liquid in the flask without interrupting the ebullition. The operation should be carried out where there is a good circulation of the air to carry off the evolved ammoniacal fumes. As the reduction is very slow the addition of the sugar solution must not be rapid. There is danger of adding too much until the operator becomes familiar with the method. If the precautions mentioned are neglected, which is usually the case, the results come out a little too low, because the air reoxidizes some of the ammoniacal cuprous solution, making it necessary to add more of the sugar to complete the reduction; that is, to completely discharge the color.

The method yields at best only approximate results, and working it subjects the analyst to the annoyance of ammoniacal fumes, unless the apparatus is complicated by the addition of a delivery tube to carry the ammonia through a window or into a fume chamber.

The reducing power of the copper in this solution depends, to some extent, on the amount of ammonia present, and from the fact that this is lost by ebullition during the performance of the test, irregularly and at different rates in different experiments, it follows that the results obtained cannot be perfectly uniform. Besides this the solution does not keep perfectly, its reducing power slowly undergoing change. However, the method has value and should be learned, because it can be rapidly worked and the results obtained are sufficiently accurate for clinical purposes.

The solution has been still further modified by substituting glycerol for the tartrate, giving what may be

called the Loewe-Pavy solution. This solution is employed as is the Pavy liquid and has the same advantages and drawbacks. For method of preparation see the appendix. It is claimed for it, however, that it keeps somewhat better. Solutions containing ammonia cannot be used for qualitative testing.

**Sugar Test by Solutions of Mercury.** — Certain solutions of mercury, like those of copper and bismuth, are readily reduced by alkaline dextrose solutions and may be employed in titration. Two such solutions are frequently used; *viz.*, Knapp's solution, containing mercuric-potassium cyanide, and Sachsse's solution, containing mercuric-potassium iodide. See the appendix for the preparation of both of these.

The solution of Knapp is frequently used in urine titration and is employed in the following manner: 10 cc. of the solution, corresponding to 25 milligrams of dextrose, are diluted with 25 cc. of water in a flask and heated to boiling. The urine, which has been previously diluted accurately with from four to nine volumes of water, is run from a burette into the hot liquid until the whole of the mercury is precipitated, which can be recognized as follows: Allow the precipitate to settle and then by means of a glass rod place a drop of the yellowish supernatant liquid on a piece of white Swedish filter-paper. Hold the paper then over an open hydrochloric acid bottle containing the fuming acid, and afterward over a beaker containing some strong hydrogen sulphide water. If the drop of transferred liquid contains even a trace of mercury this will be shown by the formation of a brown stain. In this

case it will be necessary to add more of the sugar solution, and repeat the operations until the complete reduction and precipitation of the mercury compound is accomplished, as shown by a negative result with the hydrogen sulphide test.

This method has been found to give very excellent results, but longer practice is necessary to give proficiency with it than with the other. Besides the method given above, others have been suggested for the determination of the end of the reduction, but they do not give exactly the same value for the oxidizing power of the mercuric solution.

### Color and Fermentation Methods

The methods of quantitative sugar analysis depending on comparison of colors in sugar solutions acted on by picric acid and alkali or other reagent, are neither very convenient nor accurate.

The fermentation test is sometimes applied quantitatively, but in those cases where it is the most accurate it is least necessary. With very weak sugar solutions it can only be used with the most careful regard to changes in temperature by the method referred to above. With strong diabetic urines accurate results are more readily reached, but here, by dilution, the copper solutions give the desired information more quickly and accurately.

When the saccharine urine is fermented as described and a change of specific gravity observed, the percentage of sugar is approximately given by multiplying each 0.001 lost by 0.23. For instance, if the urine be-

fore fermentation had a specific gravity of 1.032, and after fermentation, at the same temperature, a specific gravity of 1.016, we have $16 \times 0.23 = 3.68$ as the per cent. of sugar present.

### Sugar Determination by Polarimetry

The construction and method of using the polarimeter are fully explained in the author's work on "Chemical Physiology," to which the reader is referred. In practical work several forms of polarization apparatus are in actual use, but those known as "half shadow" instruments must be considered the most convenient and generally applicable.

The direct examination of urine is not always possible because of its color, and sometimes because of its slight turbidity. The best results are obtained with colorless and clear solutions. It is therefore sometimes necessary to prepare the urine by a preliminary treatment before it can be filled into the observation tubes. Diabetic urines light in color may frequently be used after simple filtration to render them perfectly clear, especially with the high-class modern instruments of the half-shadow type with which a good illumination can be secured. If the urine is much colored, so that an observation cannot be made with the shortest tube —100 millimeters in length—which can be determined by a simple trial, resort must be had to precipitation to remove part of the color. Several precipitating agents are used for clarifying sugar solutions for the polariscope. The simplest of these is a solution of basic lead acetate which produces a volu-

minous precipitate that carries down much coloring-matter. This is frequently used alone, but perhaps better combined with alum. When the basic acetate is added first and then some aluminum sulphate the mixed precipitate is flocculent and very effective in carrying down coloring-matters. Use the basic acetate of lead described in the appendix and prepare a solution of aluminum sulphate of about equivalent strength; that is, of such strength that 1 cc. will precipitate the lead of 1 cc. of the other.

Measure out 100 cc. of the urine, add 5 cc. of the lead solution and 2 or 3 cc. of the alum solution, shake well, add water to bring the volume to 110 cc. exactly, shake again and allow to stand ten minutes. Then filter through a dry filter. The filtrate will be found much lighter in color than the original and probably suitable for use. If it is opalescent pour it through the precipitate on the filter when it will be found much brighter.

There is a slight loss of sugar in this operation as some is carried down by the precipitate. The clarified solution is then filled into the polarization tube and observed in the usual manner. The result obtained must be increased by one-tenth because of the dilution of the original urine. As the precipitate formed occupies an appreciable volume when dried, the clarified solution is correspondingly concentrated and the reading from this cause would be too high. For our purpose, however, we can assume that the gain in concentration is counterbalanced by the loss of sugar inclosed with the precipitate and neglect both sources of error.

If the urine contains albumin it must be separated by coagulation and filtered out, because it rotates the plane of polarized light to the left, and would therefore make the amount of sugar appear lower.

A given volume of urine is poured into a beaker and enough dilute acetic acid is added to give a faint reaction; it is then boiled, and after standing five minutes filtered. As all albuminous bodies, however, are not precipitated by simple coagulation with acetic acid, it has been recommended to add to 100 cc. of the urine, 10 cc. of the strongly acid solution of phosphotungstic acid, already described, and filter after ten minutes. The dilution must be allowed for in the final calculation. Some coloring-matters are also removed by this treatment. The urine may contain other active substances, but in amount so small that their effect may be neglected entirely.

**Calculation of Result.**—For sodium light the formula

$$[\alpha] = \frac{100\alpha}{lc}$$

is used with the factor $[\alpha] = 52.7°$.

In this formula $[\alpha]$ = the so-called "specific rotation" of the sugar, which is a constant and must be assumed as known. $\alpha$ is the angle of rotation observed in the actual test, $l$ is the length of the observation tube expressed in decimeters, while $c$, finally, is the strength of the solution, expressed in grams per 100 cc., desired.

As we observe $\alpha$ directly we may write,

$$c = \frac{100\alpha}{l.52.7°};$$

that is, the number of grams of diabetic sugar in 100 cc. of the solution polarized is equal to the product of the observed angle of rotation multiplied by 100 and divided by the product of the length of the observation tube in decimeters multiplied by the specific rotation, 52.7°.

If in a given case we find a rotation of 10° 32.4′, with a tube two decimeters long, our formula becomes

$$c = \frac{100 \times 10.54}{2 \times 52.7^{\circ}} = 10 ;$$

that is, the concentration, $c$, is 10 grams per 100 cc.

With a decimeter tube each degree of rotation corresponds to a concentration of 1.8976. With the usual two-decimeter tube each degree indicates 0.9488 gram in each 100 cc.

The specific rotation of dextrose as obtained from urine appears to be a little higher than is that of the product made from starch.

### Other Sugars in Urine

Pathologically, traces or even larger quantities of several other saccharine bodies are occasionally found in urine. Among these we have first:

**Levulose, or Fruit Sugar.**—This is found along with dextrose in some cases of diabetes, but does not appear to occur alone.

While the recognition of levulose in the pure state or in simple aqueous solution is a matter presenting no difficulty, the certain detection of this body as it occurs in urine is by no means as readily effected. This sugar gives the reduction and fermentation tests as

described under dextrose, and therefore cannot be distinguished by these methods. Levulose, however, rotates the plane of polarized light to the left, and this property is sometimes of service in aiding the recognition. If the rotation is strongly to the left, the presence of levulose in quantity may be inferred, assuming that albumins are absent. If the quantity of sugar, calculated as dextrose, determined by polarization, is much below that found by the copper reduction method, the indication is that levulose is present with the dextrose. An exact measurement of the amounts of the two sugars when mixed in the urine is not possible with present means.

**Lactose, or Milk-sugar,** is occasionally found in the urine of nursing women. Its certain detection when in small amount presents even greater difficulties than is the case with levulose. As its rotation is right-handed the polariscopic test is of little value.

Milk-sugar is more strongly acted on by Fehling solution than is dextrose. While 1 cc. of the copper solution oxidizes 4.75 milligrams of dextrose, it oxidizes 6.76 milligrams of milk-sugar.

When a solution of milk-sugar is boiled with dilute hydrochloric acid it yields dextrose and galactose, the latter resembling dextrose in its behavior with the copper solution. The specific rotation $[\alpha]_D$, of dextrose is 52.7°, of lactose, 52.5°, while that of galactose is 81.3°. The specific rotation of a mixture of equal parts of dextrose and galactose has been found by experiment to be 67.5°, which agrees closely with the

mean of 52.7° and 81.3°. If, therefore, the rotation of urine is increased after heating with acid and neutralizing, and its copper reducing power also increased, we have data suggesting the presence of milk-sugar. Experiments to show these points with certainty must be very carefully conducted, consuming no little time in manipulation. They are, therefore, of little value from a clinical standpoint.

**Inosite, or Muscle-sugar,** has been found in urine, in diabetes, and also with albumin. There is no simple method by which it may be separated in the small quantity in which it occurs in urine.

**Other Carbohydrates.**—Traces of a body resembling erythrodextrin have been reported as occurring sometimes in urine along with sugar, or after disappearance of the same. But the origin and clinical significance of this substance are so obscure that no further attention need be given it here. The same is true of the so-called animal gum referred to by many writers. It appears to be found in small amount in all urines, and may be separated by several methods. If present in relatively large quantity, it may be recognized by boiling the urine first with dilute sulphuric acid, then neutralizing and testing with Fehling solution. By the acid treatment it is converted into a sugar-like substance which reduces alkaline copper solutions.

### Acetone

This is a substance which frequently is found in urine in small amounts. Indeed, it may be true, as

has been asserted, that it is normally always present in traces. This *physiological acetonuria* has no clinical significance. Under some circumstances, however, it may be found in larger quantity, sometimes in amount sufficient to be detected by the odor alone, which fact first called attention to it. At one time it was supposed to be related to the sugar found in urine, but it is now established that it more generally accompanies albumin and is frequently observed in many febrile conditions.

Acetone in urine is believed to be a decomposition product of albumins, or of bodies which may in turn be looked upon as resulting from proteid disintegration, as will be pointed out below. It has been shown that in health, even, it can be much increased by a diet rich in nitrogenous materials.

But, occurring as it does in fevers and in advanced stages of diabetes mellitus, a certain interest attaches to its detection, and numerous methods have been proposed by which it may be identified in small amount. Those which depend on its direct recognition in the urine are mostly uncertain. It is always safer to distil the liquid and apply the test to a portion of the distillate. Half a liter, or more, of the urine is poured in a retort attached to a Liebig's condenser, and, after addition of a little phosphoric acid, is subjected to distillation. 100 cc. of distillate will be enough. A portion of this can be taken for each test as follows:

**Legal's Test.**—Add to 25 cc. of the liquid a small amount of a fresh solution of sodium nitroprusside,

and a few drops of a 50 per cent. potassium hydroxide solution. If a ruby-red color appears which slowly gives place to yellow, and if the addition of acetic acid changes this to purple, or violet-red, the presence of acetone is indicated.

**Lieben's Test.**—This depends on the production of iodoform, and is carried out in this manner. To about 5 cc. of the distillate add a few drops of a solution of iodine in potassium iodide (the "compound solution of iodine," Lugol's solution), and then a small amount of potassium hydroxide, to marked alkaline reaction. If acetone is present a yellowish white precipitate soon appears, which, on standing, becomes crystalline and more deeply colored. The test is said to be sharper and more characteristic if ammonia is used instead of the fixed alkali. The liquid is first made strongly alkaline with ammonia, and then the iodine solution is added until the brownish precipitate formed at first dissolves very slowly. In a short time the yellowish iodoform precipitate makes its appearance. A rough quantitative measure of the amount of acetone present is given by noting the smallest volume of the distillate with which a distinct iodoform reaction can be seen. It is said that 0.0001 milligram in 1 cc. can be detected. 0.5 milligram in 10 cc. can be recognized by the nitroprusside reaction.

Creatinin gives a ruby-red color as does acetone when the nitroprusside reaction is directly applied to urine, but after adding acetic acid a green or blue color results.

**Chautard's Test.**—This depends on the production of a violet color in a solution of rosaniline hydrochloride decolorized by sulphurous acid. To prepare the reagent dissolve 1 gram of the rosaniline salt (aniline-red, fuchsin, magenta) in a liter of water. Into this solution a current of sulphurous acid gas is led until the red color is destroyed, or converted to yellow. A great excess of the sulphurous oxide must not be used. A strong solution of the acid may be used in place of the gas. To 25 cc. of this reagent add an equal amount of the acetone distillate. In presence of the latter body a reddish violet color is produced. A similar reaction is given by aldehydes and ketones in general, but in a distillate from urine acetone is indicated with practical certainty. One part of acetone in 1000 parts of distillate may be recognized by the test.

Acetone has the composition $C_3H_6O$, or $CH_3.CO.CH_3$, dimethyl ketone. It may be readily derived from the substance to be next described, acetoacetic acid, which has the formula $CH_3CO.CH_2.CO_2H$. This by distillation with acid yields acetone and carbonic acid.

### Acetoacetic or Diacetic Acid

This compound is very frequently found associated with acetone in the urine of fevers and in diabetes mellitus. While acetone may occur in very small amount normally it is believed that acetoacetic acid is always pathological. In the past few years much has been written on the subject of this substance and its clinical significance. It appears from the discussion that its presence in diabetes is of especial importance and that any increase in its amount should be

carefully followed by analytical tests. What is known as the coma of diabetes is closely associated, according to eminent authority, with the presence of acetoacetic acid in the blood.

As to the origin of this acid body there has been much speculation, and at the present time the problem is far from solution. At one time the presence of the acid was supposed to be dependent on that of β-oxybutyric acid, the two bodies being usually associated. But the diacetic acid has been found in urine free from the other, and the assumed relation does not appear, therefore, to exist. While often present in large amount, relatively, in advanced stages of diabetes the relationship of this body to the excreted sugar has never been clearly established.

Inasmuch as this acid is but slightly stable it need be looked for only in comparatively fresh urine. As it yields acetone in decomposition the tests for this body should be made first. If they show a negative result it is useless to go farther. But if positive the diacetic acid may be looked for.

**Ferric Chloride Test.**—Our main test for acetoacetic acid depends on a reaction with ferric chloride with which it strikes a red color. Normally, there is nothing in urine which gives the same reaction, so that, if on the addition of a few drops of solution of ferric chloride to fresh urine a wine-red color results, the presence of acetoacetic acid may be inferred. At the present time, however, many coal-tar products are given as remedies which yield compounds that, on elimination with the urine, give a red or purple color with

ferric chloride when added. To detect the acetoacetic acid with certainty under these conditions it is necessary to proceed with greater care. To this end add to the urine, which should be fresh, a few drops of ferric chloride or enough to precipitate the phosphates present. Filter and add a little more of the chloride. A red color indicates the acid. Divide the liquid into two portions; boil one and allow the other to stand a day or more. In the boiled portion the color due to acetoacetic acid should disappear within a few minutes, while in the other it should remain about twenty-four hours.

Acidulate another portion of the urine with dilute sulphuric acid and extract it with ether which takes up acetoacetic acid. Remove the ethereal layer and shake it with a very dilute aqueous solution of ferric chloride. The red color in the new aqueous layer should appear as before and disappear on boiling, which behavior distinguishes the acid from other substances likely to be present.

**B-Oxybutyric Acid.**—The detection of this acid in the urine by chemical methods is by no means simple, but if present in amounts not too minute it may be found by the aid of the polariscope, inasmuch as its solutions possess a strong negative rotation. In dilute solution the specific rotation is approximately $[\alpha]_D = -23.4°$. As this acid is found associated with sugar in diabetes it is necessary to destroy the sugar by fermentation before making the test. Amounts as high as 200 grams in the day's urine have been reported, but usually, where present at all, the amount is far below this, 15 to 20 grams being nearer the average.

# CHAPTER IV

## THE COLORING-MATTERS IN URINE BILIARY ACIDS

### Normal Coloring-matters

Although many investigations have been carried out on the subject of the normal urinary pigments we are yet unable to give a very definite account concerning them. This is partly due to the fact that the coloring substances exist in the urine in minute traces only, which makes their separation and recognition exceedingly difficult, and partly to another fact that some of them are easily altered or destroyed by the action of the reagents employed in their investigation. By proceeding according to different methods, physiologists have obtained very different results indicating the existence of several colors, or at any rate modifications of colors. The difficulty of detecting the normal colors in urine is sometimes increased by the presence of traces of accidental coloring-matters having their origin in peculiar or unusual articles of food consumed. Some of these will be referred to below.

It seems to be settled, however, that in health not merely one but several coloring-bodies must be present. It has not yet been found possible to separate these in the free state.

**Uroerythrin** is the name given by Thudichum and others to a common reddish coloring-matter which often precipitates with urates and other substances. It is colored green by solution of potassium hydroxide, but the color is not restored by addition of acid.

**Urobilin.**—This has been obtained as a reddish brown amorphous substance, but probably not in absolutely pure condition. It is slightly soluble in water, readily soluble in alcohol and chloroform. The *neutral* alcohol solutions are characterized by a marked greenish fluorescence which is an important means of recognition. The *acid* alcohol solutions are reddish in color, the shade varying with the concentration.

If present in more than minute traces in urine it gives characteristic absorption bands in the spectrum which have been referred to before. In acid urine the center of the dark band is near the Fraunhofer line F; in alkaline urine the center is about midway between *b* and F.

Urobilin is generally much increased in fevers and in some diseases of the liver and heart. Any cause tending to break up the red corpuscles, increases urobilin. It is not always present in sufficient quantity in normal urine to be easily recognized. If the quantity is abnormally large the following test will show it.

Add ammonia water to strong alkaline reaction and filter if necessary. Then add a few drops of solution of zinc chloride, but not enough to give a permanent precipitate. In this way a zinc salt is formed, which shows a peculiar greenish fluorescence.

Ammonia generally causes a precipitate of phosphates; hence the direction to filter. If the characteristic fluorescence fails to appear the following modification may be tried, which is sufficient to give the reaction with most urines.

Precipitate 200 cc. of urine with basic lead acetate, collect the precipitate on a filter, wash it with water and dry it. Then wash it with alcohol.

Finally, digest with alcohol containing a little sulphuric acid, and filter. The filtrate is usually fluorescent. Make it strongly alkaline with ammonia, and add solution of zinc chloride. This will give the fluorescence referred to above if but little is added, while if an excess of the zinc chloride is added, a reddish precipitate falls.

**Urophain.**—This is the name given by Heller to a substance identical with, or similar to, urobilin. Heller gives this test: Take a few cubic centimeters of strong sulphuric acid in a conical glass and pour on it, drop by drop, about twice as much urine. As the two mix, a deep garnet-red is produced.

This reaction is not, however, characteristic, as several other matters may give it.

**Urohematin** is the name given by Harley to a coloring-matter similar to the above. He applies this test: Dilute or concentrate the urine so that it is equivalent to 1,800 cc. for the twenty-four hours. Take a few cubic centimeters in a test-tube or wine-glass, and add one-fourth of its volume of strong nitric acid. No change of color can be observed if the urohematin is

present in normal amount. If more than this is present various shades from pink to red may be produced. The test should be made with cold urine, as with increased temperature darker colors result.

**Indican and its Reactions.**—Although a normal constituent of urine, indican is found greatly increased during the progress of certain diseases and becomes therefore a substance of clinical importance. It is formed along with other complex compounds in the oxidation of indol in presence of sulphuric acid. Indol is one of the common products of putrefaction, a change brought about in albuminous bodies, usually by bacterial agency. Such changes may take place in the alimentary canal, and the indol formed becomes oxidized to indoxylsulphuric acid or indican, and appears as such in the urine. The sulphuric acid necessary for the production of this body is present in combination in the system.

It was formerly supposed that this urinary indican is identical with the glucoside indican of the vegetable kingdom, from which indigo is obtained. The two substances are, however, distinct in composition and chemical behavior. The indican of urine, as stated, is the sulphuric acid combination of indoxyl, $C_8H_6N.OH$, or the potassium salt of this compound, and may be represented by the formula $C_8H_6NHSO_4$. By sublimation or treatment with oxidizing agents this yields indigo-blue or indigotin, $C_{16}H_{10}N_2O_2$.

If much indican is found it suggests that abnormal putrefaction is taking place somewhere in the body.

In diseases accompanied by the formation of putrid secretions indican usually appears in increased amount, and hence the inference derived from its ready detection. It is found in increased amount in cancer of the stomach or liver, in peritonitis, in some stages of pleurisy, in intestinal invagination (whereby the normal passage of albuminous and other food products is hindered, thus making putrefaction possible) and in other diseases.

Indican is found in normal urines in very small amount only. It may, under favorable circumstances, be detected as here given: Take about 4 cc. of pure hydrochloric acid in a test-tube and add about half as much urine, shaking well. A blue or violet color shows indican. This test depends on the conversion of the indoxyl compound into indigo, but the oxidizing action of the acid is not always strong enough to bring about the change in presence of other organic bodies in the urine.

A more generally applicable method is this: To 10 cc. of urine and the same volume of strong pure hydrochloric acid, add 2 or 3 cc. of chloroform. Then add, drop by drop, solution of sodium hypochlorite, shaking after each addition. The hypochlorite acts as an oxidizing agent, liberating the coloring-matter, which is then taken up by the chloroform. The oxidation must not be carried too far; that is, too much hypochlorite must not be added, as it would then destroy the color as fast as formed. In fact, small traces of the product sought might be completely overlooked in the process, as the hypochlorite is a very active

oxidizer, the effect going far beyond the production of indigo. It has therefore been proposed to use nitric acid as the oxidizing agent. The urine is boiled with an equal volume of hydrochloric acid and then a few drops of nitric acid are added. The mixture is cooled and to it a little chloroform is added and well shaken. In presence of indigo the blue color appears. Bromine water in small amount may be employed in the same manner.

Albumin must be separated by coagulation before applying either of these tests, as it develops a blue color with hydrochloric acid. The amount of indican normally present in urine is said to vary between 5 and 20 milligrams daily. The chloroform layer in the bottom of the test-tube in the above test shows roughly by the depth of color developed the amount of indican present. It is necessary to use good hypochlorite for that test as with a weak solution the oxidation may fail to take place.

### Abnormal Coloring-matters

In disease several other coloring-matters may appear in urine, the most important of which are those of the bile and blood.

As abnormal colors must be classed, also, many products taken into the stomach with the food or as remedies and which appear directly in the urine or give rise to marked coloration on the addition of reagents.

#### Biliary Coloring-matters

These are found in the urine in jaundice and may be traced to the stoppage of the bile ducts of the liver

as in common jaundice and to other causes having no connection with a disorder of the liver. The appearance of these coloring-matters in the urine is therefore a symptom of different diseases, although perhaps most commonly associated with an abnormality in the flow of the bile. Jaundice may sometimes be traced to a disintegration of the red corpuscles in the blood and consequent liberation of derived coloring-matters.

Biliary urine has generally a characteristic greenish yellow color sometimes tinged with brown. The froth from such urine is readily recognized by its yellow color, which is often a sufficient test in itself. Among the chemical tests the following are the best known.

**Gmelin's Test.**— This is easily performed and depends on the oxidation of bilirubin, the pigment commonly present in fresh jaundice urine, by nitrous acid. Pour in a test-tube about 5 cc. of the urine under examination and by means of a pipette introduce below it an equal volume of strong nitric acid mixed with nitrous. This should be carefully done so as to avoid mixing the liquids much. At the junction of the two liquids, if bile is present, several colored rings appear of which the green due to biliverdin is most characteristic. The bands or rings appear above the acid in this order, yellowish red, red, violet, blue, and green. The last is essential. It must be remembered that nitric acid gives the other colors at times with urine free from bile, but green is characteristic of the latter.

Fleischl modified this test by mixing the urine with a strong solution of sodium nitrate and then adding

strong sulphuric acid carefully. This settles below the urine and decomposes the nitrate at the point of contact liberating the necessary nitric and nitrous acids for the oxidation as before. This method is a very good one.

In another modification, urine is dropped on a plaster of Paris disk and then a few drops of the oxidizing mixture of nitric and nitrous acids is placed in its center. The same play of colors appears as before.

**Trousseau's Test.**—Add to some urine in a test-tube a few drops of tincture of iodine, allowing the iodine to float on the urine. If bile pigments are present a green color is produced when the iodine touches the urine, and persists some hours. Care must be taken to avoid using an excess of the iodine if the liquids are allowed to mix. In this case with the proper amount of the tincture the whole urine appears green.

**Heller's Test.**—Take 5 or 6 cc. of pure strong hydrochloric acid in a conical glass and add enough of the urine to give it a faint color on mixing. Now add pure nitric acid by means of a pipette so as to bring the latter under the mixture of hydrochloric acid and urine. The colored rings appear as in the Gmelin test and on shaking can be followed through the liquid.

**The Detection of Traces.**—To 100 cc. of the urine add 10 cc. of pure chloroform and shake gently until the latter is colored. By means of a pipette withdraw a small part of the chloroform and mix it in a test-tube with 10 cc. of strong pure hydrochloric acid. Add

nitric acid as in the other tests and shake. With bile present, the oxidation colors appear slowly in the chloroform, the green being the deciding tint.

**The Diazo Reaction.**—Ehrlich and others have called attention to the behavior of many urines with solution of diazobenzene sulphonic acid which often has importance in diagnosis. Normal urine treated with a weak solution of this reagent shows no marked change, but in several pathological conditions after adding the acid and saturating with ammonia a deep carmine- or scarlet-red color appears, followed by greenish or violet precipitation.

This reaction depends on the combination of the sulphonic acid of diazobenzene with some aromatic compound found in the urine in pathological condition. At one time it was supposed to have special significance in the diagnosis of typhoid fever, but it now appears that in many diseases of the intestinal tract the urine receives traces of complex aromatic products of bacterial origin which respond to the test. It has, therefore, general, rather than special, significance.

As the diazobenzene sulphonic acid is not very stable, it is not convenient to use, and a reagent is made which, in its application, is its chemical equivalent. The reagent is prepared by dissolving 1 gram of sulphanilic acid in 200 cc. of water with the addition of 10 cc. of pure hydrochloric acid. Another solution is made by dissolving 1 gram of sodium nitrite in 200 cc. of water. To make the test take 50 cc. of the first solution, add 5 cc. of the nitrite solution

and then 50 cc. of urine. Ammonia is then added in sufficient quantity to impart a strong alkaline reaction after thorough shaking. A scarlet-red color is the result if the urine in question contains the abnormal products referred to.

**Melanin.** — In the urine of patients suffering from melanotic cancer a dark color sometimes appears which may become almost black on exposure to air. Treatment of the urine with oxidizing reagents increases the effect; with bromine water a yellowish color is first given followed by black. The coloring substance may be even thrown out as a brownish black sediment. In urine containing this body, to which the name melanin has been given, yellowish brown precipitates are produced by the addition of baryta water and basic lead acetate, but the coloring-matter itself has never been isolated in pure condition. Its recognition may have diagnostic value in cases where the melanotic condition may not otherwise be suspected.

### Blood Coloring-matters

As these appear in the urine they may be derived from different sources. We may have, first, color due to the presence of blood corpuscles themselves sometimes in nearly fresh condition. There may be enough blood present to impart to the urine a marked red color and it may be derived from the kidney, bladder, urethra, or other part of the urinary tract. In blood from a fresh lesion the corpuscles usually appear in clearer outline than is the case when they have remained long in contact with the urine.

The presence of blood may be detected by several methods. The corpuscles are often easily recognized by the microscope in the sediment deposited when the urine is allowed to stand, as will be explained in a following chapter. Then we can make use of the spectroscope, by which means the characteristic absorption bands of oxyhemoglobin are detected, as explained in works on chemical physiology. If urine containing blood is treated with a few drops of ammonium sulphide and very gently warmed, the spectrum of reduced hemoglobin is given.

Sometimes the coloring-matters alone without the corpuscles can be found. This is the case when the latter become disintegrated, the more stable and soluble hemoglobin passing into solution while the stroma disappears by decomposition. The condition in which blood itself is present, and can be recognized by the microscope, is known as *hematuria*, while the condition characterized by the presence of the coloring-substance only is called *hemoglobinuria*. Urine containing the products of blood decomposition is often brown or smoky colored.

In urine, hemoglobin frequently undergoes two decompositions. It may become converted into *methemoglobin*, or it may suffer a complete modification, breaking up into hematin and a body resembling globulin. Hematin is best recognized by spectroscopic examination, as it gives a spectrum different from hemoglobin. This modified product is said to occur in urine in cases of poisoning by hydrogen arsenide.

The following are the best chemical tests for the recognition of these bodies:

**Heller's Test.**—Treat the urine with solution of sodium or potassium hydroxide, and heat to boiling. This produces a precipitate of the earthy phosphates which in subsiding carry down coloring-matters. If a precipitate does not separate readily it may be hastened by adding two or three drops of magnesia mixture. Hemoglobin, when present, is decomposed by this treatment with separation of hematin, which in turn settles down with the phosphates, imparting a red color to the precipitate.

**Struve's Test.**—Make the urine slightly alkaline with sodium hydroxide solution, and then add enough solution of tannic acid in acetic acid to change the reaction. If hemoglobin is present a dark brown precipitate of hematin tannate settles out. The test is a good one, and easily performed, but is not sufficiently delicate for the detection of small traces of hemoglobin directly. By collecting the precipitate on a filter and washing it, it may be used for two confirmatory tests. One of these depends on the formation of hemin crystals and is made in this manner: Place a small portion of the precipitate on a microscopic glass slide and add a minute crystal of sodium chloride. Then add a large drop of glacial acetic acid and cover with a cover glass. Warm very gently over a small flame. When the acid, salt, and precipitate have become thoroughly mixed allow the slide to cool. Small rhombic

crystals of hemin should now appear, which are best seen under a microscope.

The washed precipitate may also be ashed and used for an iron test. The ash should be dissolved in a little pure hydrochloric acid in a porcelain dish and tested by the addition of potassium ferrocyanide and ferricyanide to give the well-known reaction. This test presupposes purity and freedom from traces of iron in the reagents used.

**Almen's Guaiacum Test.**—In a test-tube mix equal volumes of fresh tincture of guaiacum and ozonized turpentine,—2 or 3 cc. of each will suffice. The mixture, if made of proper materials, must not show a green or blue color after thoroughly shaking. Now add a few cubic centimeters of the urine to be tested, a drop at a time, and agitate after each addition. If hemoglobin is present it causes the oxidizing material of the ozonized turpentine (probably hydrogen peroxide) to act on the precipitated guaiacum resin, imparting to it first a greenish, and finally a blue color. Old and alkaline urine must be made faintly acid before performing the test. Pus in the urine gives a somewhat similar reaction, and a few other bodies, very seldom present, interfere. The test is very delicate, and if it gives a negative result it is safe to conclude that blood is absent.

### Vegetable and Other Colors

It has long been known that many peculiar coloring-matters enter the urine from substances taken as reme-

dies and sometimes as food. A few of the more common of these colors will be mentioned here.

**Chrysophanic Acid.**—This complex organic acid is found in the root of several kinds of rhubarb, in senna leaves, in certain lichens, and elsewhere. After the administration of any of these substances the urine becomes more highly colored, being a brighter yellow if acid and yellowish red when made alkaline. When phosphates are precipitated by addition of alkali they appear red in presence of chrysophanic acid, as they do with blood. But the latter can be easily distinguished by the other tests already given. Urine containing this acid is further distinguished by giving a red precipitate with solution of lead acetate.

**Santonin.**—This crystalline principle is found in the unexpanded flowers of Levant wormseed, and when administered as a remedy produces a characteristic change in the color of the urine. The color becomes a deep yellow which turns red with alkalies, as in the case of chrysophanic acid. If the colored alkaline urine is shaken with amyl alcohol the coloring-matter from the santonin leaves the urine and passes into the alcohol, but the color from chrysophanic acid is only very slightly soluble in amyl alcohol and remains with the urine when the same treatment is applied.

**Salicylic Acid.**—The urine of persons taking this substance has usually a grayish smoky tinge which becomes blue on addition of solution of ferric chloride if more than traces are present.

Salicylic acid is excreted in the free state or as a salicylate of sodium or potassium mainly; a small portion seems to pass into other compounds. But as the iron reaction is very delicate minute amounts of the free or combined acid can be found. Enough ferric chloride must be added to be in excess of what would combine with the phosphates present, otherwise a sharp reaction may not be secured.

**Phenols.**—Several phenol bodies as carbolic acid, hydroquinol, resorcinol, pyrocatechol and others sometimes find their way into the urine, to which they impart a dark color on standing exposed to the air. This change of color is said to be due to the formation of oxidation products of hydroquinol. From urine darkened in this manner phenols have been recovered by making acid with sulphuric acid and then distilling with steam.

Some of these phenols, in traces, are undoubtedly normal urinary constituents, but pathologically they may appear in increased amount, and also after administration of various aromatic remedies. The external application of common phenol is followed by the appearance of traces in the urine, which is disclosed usually only after the latter has stood some time exposed to the air. Many reactions are given in the books for the recognition of traces of phenol, but as a rule they cannot be applied directly to urine. For detection here a liter may be distilled after the addition of 25 cc. of strong sulphuric acid. To a portion of the distillate add enough bromine water to impart a yellow-

ish color. In presence of phenol a light yellowish precipitate of tribromphenol appears. To a second portion of the distillate add a few drops of ferric chloride solution; with phenol this gives a purple color. The same reaction is given by other phenols and by salicylic acid. To separate the latter a portion of the distillate is made alkaline with sodium carbonate and the solution so obtained is shaken with ether, the operation being repeated several times. The salicylic acid is held, while phenols pass into the ethereal solution. After evaporating the ether and taking up with water the tests for phenols may be made.

**Other Colors.** — Blueberries, carrots, and several other common vegetable foods give deep color to the urine. It is not always possible to recognize the coloring-substances in these cases. Such urine usually becomes yellow with acids and reddish with alkalies. It is occasionally possible to identify the color by means of the spectroscope, as the absorption spectra of some of these products have been studied.

### The Detection of the Bile Acids

It sometimes happens that the physician desires information regarding the presence of the biliary acids as well as the pigments in the urine. This information, however, is not easily secured because there is no simple test which can be applied directly to the urine which will give a certain indication of the presence of these acids. They must first be separated from the large amount of other substances present, which can be done in this way (Neukomm):

Evaporate 300 to 500 cc. of urine nearly to dryness; extract with ordinary alcohol, evaporate this solution, and extract the residue with absolute alcohol.

Evaporate this and take up the new residue with water. Precipitate the solution by lead acetate, *avoiding excess;* allow to settle, wash the precipitate with water on a filter, and dry in folds of bibulous paper. This leaves an impure lead salt of the acids. Extract it with hot alcohol, and filter; add sodium carbonate to the filtrate, evaporate to dryness and extract the sodium salt, thus formed, with absolute alcohol. Evaporate again, add some water and apply the Pettenkofer test, as follows:

To the solution add one or two drops of a 20 per cent. cane-sugar solution, and then some strong sulphuric acid, slowly to avoid heating.

It is best to immerse the test-tube in water to keep the temperature below 60° C. As the acid mixes with the liquid a violet or purple color is produced. It has been shown that this, like the *α*-naphthol test for dextrose is a furfural-reaction, the furfural formed from the mixed sugar and acid combining with the acids of the bile. It has even been proposed to use a dilute solution (one-tenth per cent.) of furfural instead of the sugar in the test.

Kuelz recommends to evaporate the solution on a water-bath to dryness, to moisten the residue with a drop of dilute sugar solution, and then with a drop of the strong acid. The color appears almost immediately, but can be sharpened by heating the evaporating dish a few seconds on the water-bath.

Applying either of these tests directly to urine is unsafe, as the coloring, and other, matters present would intrefere very much with the reaction.

# CHAPTER V

## DETERMINATION OF URIC ACID. HIPPURIC ACID

Uric acid, $C_5H_4N_4O_3$, occurs normally in urine combined with sodium, potassium, magnesium, or ammonium. The absolute amount excreted daily is small but quite variable, depending on many conditions not well understood. In health the amount passed daily seems to vary between 0.2 gram and 1 gram. These limits may not be correct, however, as many of the older determinations were made by inaccurate methods.

Regarding the clinical significance of variations in the amounts of uric acid passed, our knowledge is still very defective. It is generally held that there is a considerable increase in the excreted uric acid in fevers and in diseases characterized by diminished respiration and consequently imperfect oxidation. In leucemia there is a pronounced and characteristic increase of uric acid. Certain writers have attempted to connect a decreased elimination of uric acid with an accumulation of the same in the blood, giving rise to numerous disorders of which *gout* may be mentioned as one in which the connection has been, apparently, clearly shown. Great variations in the excreted uric acid seem to be characteristic of a train of disorders, rather than of a single one.

From recent investigations it appears that the ratio of excreted urea to uric acid is in health not far from

35 : 1, and that variations in this ratio are of greater moment than are variations in the absolute amount of the acid. Both must be considered as normal end products of nitrogenous metabolism, contrary to the older view that uric acid is the antecedent of urea, and that the amount of the former found in the urine represents merely that which failed to be completely oxidized. A marked change in the above ratio, 35 : 1, by increase of the uric acid is characteristic of a condition which is somewhat indefinitely called the uric acid diathesis.

In the recognition of uric acid the following points may be noted : When present in large amount it frequently precipitates from the urine in the free form, or as acid urates which have a yellowish color. When the amount present is small it may be found by acidifying with hydrochloric acid and then allowing the urine to stand some hours in a cool place; uric acid crystals separate. In mixed sediments it may be recognized by this test:

**Murexid Test.**—Throw the sediment on a filter and wash once with water. Place the residue in a porcelain dish, add a drop of strong nitric acid, and evaporate to dryness on the water-bath. A yellow or brown mass is obtained, and this touched with a drop of ammonia water turns purple.

Unless the uric acid or urate is present in the sediment in fine granular form its recognition by the microscope is very simple. Illustrations of the forms of uric acid and certain urates are given in the chapter on the sediments.

### The Amount of Uric Acid

For the determination of the amount of the acid in the urine we have the choice of several methods, not one of which is very convenient or of the greatest accuracy. The first of these depends on the fact referred to above, that hydrochloric acid liberates uric acid from its combination, precipitating it in crystalline form.

**Precipitation Test.**—Measure out 200 cc. of urine and add to it 20 cc. of strong hydrochloric acid. Mix thoroughly and set aside in a cool place for about forty-eight hours. At the end of this time collect the reddish-yellow deposit on a weighed filter, wash it with a little cold water, dry, and weigh. Not over 30 or 40 cc. of water should be used in the washing. The precipitated uric acid is not pure, holding coloring and other substances which increase its weight. On the other hand, it is soluble to some extent even in cold acidulated water so that not the whole of it is obtained on the filter and a correction must be made. It is usually recommended to add to the weight obtained 4.8 mg. for each 100 cc. of filtrate and washings.

If the urine under examination contains albumin, the latter must be coagulated by heating with a drop or two of acetic acid and filtered out, before the test is made. If the urine is very cold to begin with and has a sediment of urates, the latter must be brought into solution by warming before beginning the test. To prevent precipitation of phosphates during the

warming a few drops of hydrochloric acid may be added. This method is at best only a rough approximation, but is the one by which most of our results have been obtained. The following gives better results:

**Salkowski-Ludwig Method.**—The determination here is based on the fact that uric acid gives a very insoluble precipitate with ammoniacal solution of silver nitrate, from which precipitate, after filtration and washing, the acid may be readily separated, brought into *concentrated* solution, reprecipitated and weighed.

In using the method the following solutions are required:

(*a*) **Ammoniacal Silver Nitrate.**—Dissolve 25 grams of silver nitrate in 100 cc. of distilled water, add ammonia water until the precipitate which appears at first is completely redissolved, leaving a clear solution. Make this up to 1000 cc. with distilled water and keep in a dark bottle or away from the light.

(*b*) **Magnesia Mixture.**—Made as described in the appendix. It must be strongly alkaline and clear, or nearly so.

(*c*) **Solution of Potassium or Sodium Sulphide.**—The pure crystals of sodium sulphide obtained from dealers in chemicals may be used by dissolving 25 to 30 grams ($Na_2S.9H_2O$) in 1000 cc. of distilled water. A solution may be made, also, by dissolving 10 grams of pure sodium hydroxide in 1000 cc. of water, and converting this into sulphide, which is

done as follows: Divide the solution into two equal portions. Saturate one thoroughly with hydrogen sulphide and to this then add the other half. Keep in a glass-stoppered bottle, the stopper paraffined.

To make the test, measure out 200 cc. of the urine and transfer to a beaker. Add 20 cc. of the silver solution, (*a*), to an equal volume of the magnesia mixture, (*b*), and then ammonia enough is added to clear up any precipitate which forms. This clear mixture is now poured into the urine in the beaker and the whole well stirred. A precipitate of silver urate forms along with silver and earthy phosphates. The excess of ammonia prevents the precipitation of silver chloride. Silver urate is quite insoluble in ammonia; it is gelatinous alone and does not settle very well but the phosphate precipitate corrects this difficulty to some extent. The beaker is allowed to stand at rest about an hour, after which the contents are filtered and the precipitate washed with weak ammonia on the filter. To do this the ammonia is sprayed into the beaker from a wash-bottle and rinsed around thoroughly. This is done several times, the liquid being poured on the filter. Where available a Gooch crucible serves admirably for the collection of the precipitate as the filtration is slow on paper without aspiration. It is not necessary to remove any of the precipitate which clings to the beaker, as will be seen. When the washing is complete transfer the precipitate and filter-paper, or asbestos if the Gooch crucible is used, back to the beaker and pour over it a boiling mixture

of 20 cc. of the sulphide solution, (*c*), and 20 cc. of distilled water. Stir up thoroughly, allow to stand some time and then add 50 cc. of boiling water. Place the beaker on a sand-bath or gauze and bring the contents to boiling, stirring continually. Keep hot some minutes and then allow to stand until cold, the precipitate being stirred meanwhile occasionally.

The treatment with the sulphide solution decomposes the silver urate with precipitation of black insoluble silver sulphide, the uric acid remaining in solution as soluble urate. The cooled liquid is filtered into a porcelain dish, and the precipitate washed with warm water, the washings going also into the dish. Enough hydrochloric acid is now added to combine with all the bases present and liberate the uric acid, which is the case when the liquid becomes acid in reaction. It is now slowly evaporated to a volume of about 10 cc., best on a water-bath, and then allowed to stand an hour for the complete separation of the uric acid. This is then collected on a weighed Gooch crucible, the crystals being transferred gradually by aid of the filtered liquid. When the crystals are on the asbestos they are washed with a little acidulated water several times. The crucible is then dried at 100°, put back in the funnel and treated with a small amount of pure carbon disulphide to remove traces of sulphur separated on decomposing the alkali sulphide. Finally, wash with ether, dry at 100° C. and weigh. The results are always a little low, but fairly regular.

As the acid is finally precipitated from a very small volume of liquid and but little water is used in wash-

ing, no correction need be made for solubility, as in the first process described. While simple enough in principle and easily carried out considerable time is required for the performance of all the operations involved in the method.

The following method is free from this objection and is equally accurate:

**Haycraft Method.**—This depends on the precipitation of the uric acid, as silver urate, and its subsequent titration by standard solution of ammonium thiocyanate. The following solutions are required:

(*a*) **Ammoniacal Solution of Silver Nitrate.** — Dissolve 5 grams of crystals in 100 cc. of water and then add enough ammonia water to give the solution a strong alkaline reaction. Make up to 200 cc. with the ammonia.

(*b*) **Ammonium Thiocyanate.**—This solution is made as described later for the determination of chlorides in urine by the Volhard method. It is given just one-fifth the strength there described and may be made by diluting 100 cc. of that solution to 500 cc. in a measuring flask.

(*c*) **Ammonium Ferric Sulphate** (ferric alum).— Saturated solution as indicator. Described under the chlorine test.

It has been shown by Dr. Haycraft that silver combines with uric acid in constant and definite proportion; *viz.*, one atom of silver to one molecule of the acid, or 107.9 parts of silver to 168.2 of the acid, giving the formula $AgC_5H_3N_4O_3$.

This precipitate dissolves in dilute nitric acid and if the solution so obtained is treated with the ammonium thiocyanate the following reaction takes place:

$$AgC_5H_3N_4O_3 + NH_4SCN = AgSCN + NH_4C_5H_3N_4O_3.$$

From this it follows that 1 cc. of a fiftieth normal, ($\frac{N}{50}$), solution of the thiocyanate liberates and indicates 0.00336 gram of uric acid.

It is fully explained under the chlorine test that if a solution of a thiocyanate is added to a solution of a silver salt containing nitric acid and ferric sulphate, a complete reaction takes place between the thiocyanate and silver before the characteristic reaction between the former salt and the ferric compound appears. In other words, the thiocyanate and the silver combine first and then any further amount of thiocyanate added unites with the iron, producing a red color (of ferric thiocyanate) *indicating* the completion of the first reaction.

With these general explanations the process will now be understood.

Measure out 50 cc. of the urine and warm it gently if it contains a sediment of urates. Add 3 to 4 grams of pure sodium bicarbonate and then ammonia enough to give a strong alkaline reaction. This may give a precipitate of phosphates which need not be heeded. Next add 5 cc. of the silver solution, (*a*), and mix thoroughly. This produces a precipitate of silver urate along with the bulky phosphates thrown down by the ammonia. Allow to stand half an hour and then filter. A paper filter and funnel may be used in

the usual manner, but much better results are obtained by the use of the Gooch crucible and asbestos with aid of an aspirator. Rinse the sides of the beaker thoroughly with weak ammonia and pour this on the precipitate in the funnel or crucible. Continue the washing of the precipitate with weak ammonia water until all traces of silver are washed out, as may be shown by allowing a few drops of the filtering washings to fall into some dilute hydrochloric acid in a test-tube. The washing is complete when a cloudiness is no longer obtained in this test.

Now pour some pure dilute nitric acid into the beaker in which the precipitation was made, and which was washed free from silver by the ammonia, and shake it around until any traces of the silver urate precipitate are dissolved. Put the funnel or Gooch crucible over a clean receptacle and pour this acid liquid on the precipitate. Silver urate dissolves completely in dilute nitric acid, and enough of this is added, a little at a time, to bring about complete solution. It now remains to titrate the silver in this solution. To this end add 5 cc. of the ferric alum solution, and if the mixture is not clear and colorless, about 2 cc. of pure strong nitric acid. Then from a burette run in the thiocyanate, (*b*), a little at a time, shaking after each addition until a faint red shade of ferric thiocyanate becomes permanent. Toward the end of the titration a red appears as each drop of liquid from the burette falls into the silver solution below, but this color fades out on shaking and does not persist until the last particle of silver has been

taken up by the thiocyanate. Supposing now that 15 cc. of the latter solution are required to reach this point we have $15 \times 0.00336 = 0.0504$ gram as the amount of uric acid in the 50 cc. of urine taken. A volume as large as this would seldom be required; 5 to 10 cc., corresponding to 16.8 to 33.6 milligrams, is usually sufficient.

The method gives results which are a little too high as the silver carries down traces of other bodies as well as uric acid; but the error is not great enough to interfere with the practical application of the process where the best obtainable results are desired. The washing of the precipitate of silver urate is the point which requires the greatest care. A little practice will show how this can be best done.

**Fokker-Hopkins Method.**—It has long been known that uric acid may be very completely precipitated from urine in the form of acid ammonium urate by the addition of an ammonium salt. The method of precipitation as first described by Fokker was not satisfactory, and was modified by Salkowski. More recently it has been improved by Hopkins, and in this form may be carried out as follows: Add to 100 cc. of urine enough pure, finely granular or powdered ammonium chloride to completely saturate it. About 30 grams of the salt will be necessary for this. The urine must be well stirred as the chloride is added to facilitate its solution. When no more will dissolve the solution is allowed to stand about two hours and should meanwhile be stirred occasionally. The precipitate

which settles is collected on a small filter and well washed with a saturated solution of ammonium sulphate. In this washing the precipitate is freed from some coloring, and other, matters and is then ready for further treatment. Two methods are available for rapid work. In the first the filter with the precipitate is placed over a beaker or flask, and through a hole made in the bottom of the paper with a glass rod the precipitate is washed down into the receptacle. This can be easily done with the aid of a fine jet from a wash-bottle. Use about 100 cc. of water and to the turbid liquid add now 20 cc. of pure colorless sulphuric acid. This produces a high temperature and dissolves the uric acid. The amount of the latter is now found by titration with twentieth normal permanganate solution (1.581 grams of $KMnO_4$ per liter) as follows: The permanganate solution is contained in a burette and without delay is run into the hot uric acid solution. A reduction of the reagent, with loss of color, follows. When the uric acid is fully oxidized a farther addition of permanganate leaves a pink tinge in the liquid. The addition of the standard reagent from the burette should cease as soon as a pink tinge is reached, which is permanent two seconds after good shaking. By waiting a longer interval the color fades and more solution must be added from the burette. If the reaction is stopped with the first decided tinge obtained, as explained, for each cubic centimeter of permanganate used from the burette, 3.75 milligrams of uric acid may be calculated as present. In illustration, suppose we start with 100 cc. of urine and pre-

cipitate, wash and dissolve as described. If now we run 12.5 cc. of the twentieth normal permanganate solution into the hot uric acid solution to obtain the pink color, the amount of this acid present is $12.5 \times 3.75 = 46.87$ milligrams in the 100 cc. Urine contains traces of other bodies which are precipitated with the uric acid, but in amount so small that their effect may be practically neglected.

Instead of oxidizing the uric acid precipitate with permanganate solution it may be titrated by means of weak standard alkali solution, preferably twentieth normal. When this is to be done it is best to start with 200 cc. of urine and precipitate and wash as before. The precipitate is boiled up with 30 cc. of twentieth-normal hydrochloric or sulphuric acid and enough water to make about 200 cc. Then two drops of a weak methyl orange solution are added and finally, from a burette, the twentieth-normal alkali until the reddish pink color changes to orange-yellow. Part of the acid added is used in decomposing the acid ammonium urate, and is therefore a measure of the latter. The alkali run in measures the excess of hydrochloric acid, as uric acid is neutral toward methyl orange. Therefore, subtract from the 30 cc. of acid the volume of alkali required to give the final reaction; the remainder measures the alkali actually required for the uric acid. For each cubic centimeter of alkali calculate 8.4 milligrams of uric acid. In illustration, suppose we precipitate 200 cc. of urine, collect and wash the precipitate and dissolve as described, adding 30 cc. of twentieth-normal acid.

If now we run in 13.5 cc. of twentieth-normal alkali we have 30 — 13.5 = 16.5 cc. of alkali actually required for the uric acid. Then, 16.5 × 8.4 = 138.6 milligrams in 200 cc.

In a following chapter something will be said about the occurrence of uric acid sediments in urine. Under certain conditions a large part of this acid present may separate in the free form or in combination as slightly soluble urate, readily recognizable by the microscope.

### Hippuric Acid

This acid, which is benzoyl-amidoacetic acid, $C_9H_9NO_3$, is found in very small amount normally in human urine, and is the chief nitrogenous product in the urine of the herbivora. It is increased in human urine by a diet of aromatic vegetable substances, but is seldom abundant enough to have clinical importance. The amount excreted varies usually between 0.1 gram and 1 gram daily. Even larger amounts have been reported, but the excretion seems to be connected with the consumption of unusual fruits or vegetables. The effect of cranberries is especially characteristic here.

It is also known that benzoic acid and benzoates taken internally become changed in the body to hippuric acid and are so excreted. Under such circumstances the percentage amount may become relatively very large, even as much as 1.5 to 2 per cent.

For the detection of hippuric acid in urine it is best to take 1000 to 1500 cc. and make slightly alkaline, if acid, with sodium carbonate. Filter and evaporate

the filtrate nearly to dryness. Extract the residue several times with small portions of alcohol, evaporate the alcohol and treat the aqueous solution left with enough hydrochloric acid to impart a sharp acid reaction. Pour this solution into a separatory funnel and shake out with acetic ether five or six times. This dissolves the hippuric acid. The different portions of acetic ether are united and washed in the funnel with water. The so-purified ethereal solution is evaporated slowly to deposit the hippuric acid. If traces of fat appear to be present wash this residue with petroleum ether. Then dissolve the remaining acid in hot water, filter and allow the solution to evaporate spontaneously, or at a temperature not above 50°, to crystallization. Hippuric acid may be recognized by the microscope, as shown later, or by this chemical test. To some of the crystalline product in a dish add a little strong nitric acid and evaporate to dryness. By heating now carefully to a higher temperature a strong odor of nitrobenzene is developed. In this reaction nitrobenzoic acid is produced from the hippuric acid and at a higher temperature yields carbon dioxide and nitrobenzene.

# CHAPTER VI

## UREA

Urea, $CO(NH_2)_2$, is the most important nitrogenous substance excreted in human urine. A large part of the nitrogen of our food is normally converted into urea for elimination from the body, but how this conversion takes place, or where, is not definitely known. It has been shown that in the liver it may be produced from ammonium carbonate and certain other comparatively simple bodies, but the connection with the antecedent and much more complex proteid bodies is quite obscure. Experiments which have been carried out by physiologists to determine the part played by the kidneys in the elimination of urea have led to very contradictory results. It has been found that the synthesis of hippuric acid from benzoic acid and glycocoll may be effected in the extirpated kidney, but attempts to form urea in an analogous manner from blood charged with ammonium carbonate led only to negative results, from which it would appear that the function of the kidneys, as far as the elimination of urea is concerned, is merely a mechanical one. Other investigations, however, have led certain observers to the view that the cells of the kidney are active in the formation of urea from products coming from the disintegration of elements of the blood, if

not from ammonium salts. After extirpation of the kidney there is observed an accumulation of urea in the blood of the living subject, which is a point not without importance, as it shows that this organ can *not* be the only seat of the reaction of urea production.

Not far from 85 per cent. of the nitrogen consumed as food is excreted as urea, but the absolute amount of the latter passed in a day is exceedingly variable. In the urine of the average man it is between 30 and 40 grams while in the urine of women it is less. The variations depend mainly on the diet, the urea being highest with a diet rich in meat, eggs, beans, peas, and similar vegetables, and low with a diet of fruits, bread, and potatoes. The *percentage* amount of urea depends further on the volume of the urine passed in a day and may vary from a change in the amount of water consumed and also from different losses by perspiration. The percentage amount of urea depends also on the time when the urine is voided. A determination of value should therefore be made on the mixed urine of the twenty-four hours.

It is usually assumed that 2 per cent. is the average amount excreted in health, but this is probably low. While the variations from this mean are great in health, they are much more marked in pathological conditions.

Urea is increased in total amount, although it may be diminished in percentage in diabetes mellitus and insipidus and also in fevers. It has been found to be increased in cases of poisoning by heavy metals, but why is not clearly demonstrated.

Clinically, the increase in diabetes and fevers is of the greatest interest because we have here evidence of increased consumption of the nitrogenous tissues of the body. A diminished elimination of urea has been observed in acute yellow atrophy of the liver and in other diseases of that organ. This has been taken to indicate that the liver may be the place of formation of urea. In cases of malnutrition in general the absolute and percentage amount of urea may be greatly diminished.

A marked decrease has been observed, also, in diseases involving structural changes in the tubules of the kidney as in parenchymatous nephritis, and this suggests again the relation of the kidney to the formation of urea.

It has been observed that an increase in the body temperature, as from taking hot baths, is followed by an increased elimination of urea. This in turn seems to be compensated for by a period of diminished elimination. It is also a matter of practical experience that the administration of many inorganic salts causes an increase in the quantity of urea passed. The same observation has been made with a number of alkaloidal salts, but the results of experiments are hardly definite or full enough, as yet, to warrant the drawing of final conclusions.

By some authorities ammonium carbonate is looked upon as the immediate forerunner of urea, which latter is formed by splitting off of water from the former:

$$(NH_4)_2CO_3 = CO(NH_2)_2 + 2H_2O.$$

As bearing on this it has been observed that relatively large quantities of ammonium carbonate and organic ammonium salts, when taken as remedies, do not increase the alkalinity of the urine, but leave the body as urea. It has been supposed that this change takes place in the liver.

**Recognition of Urea.**—Because of its extreme solubility urea cannot be easily obtained by evaporation of urine. It has been shown, however, in an earlier chapter that by concentrating the urine slowly to a small volume—to one-third or one-fourth—cooling and adding strong nitric acid, a crystalline precipitate of plates of urea nitrate separates, which is characteristic. From this precipitate pure urea can be obtained.

Clinically, this test has no importance, as we are concerned only with a measurement of the amount of urea. This determination can be made in several ways, but in actual practice we employ three essentially different methods. The first depends on the fact that solutions of urea precipitate solutions of certain metals in a definite manner, from which a volumetric process has been derived. The second depends on the fact that solutions of certain oxidizing agents decompose solutions of urea with the liberation of its nitrogen (and carbon dioxide) in gaseous form. From the known relations between weight and volume of the gas, and weight of nitrogen and weight of urea, the absolute amount of the latter may be calculated. The third method depends on the fact that when the urea of urine is decomposed into water, carbon dioxide and

nitrogen, its specific gravity is decreased in a manner empirically determined. The loss in specific gravity bears a certain relation to weight of urea present.

## Determination of Urea

**Liebig's Method.**—We have here the oldest, and, in many respects, the best of our processes for the titration of urea. The principle involved in the method is this: When a solution of mercuric nitrate is added to a solution of urea a white precipitate forms and settles out. By working with solutions of a certain definite concentration it has been found that the reaction between the mercury and urea takes place in constant proportion and according to this equation:

$$2CON_2H_4 + 4Hg(NO_3)_2 + 3H_2O = 2CON_2H_4.Hg(NO_3)_2.3HgO + 6HNO_3$$

This precipitate contains 10 parts of urea for every 72 parts of HgO. 72 grams of HgO dissolved in $HNO_3$ should precipitate, therefore, 10 grams of urea.

The same solution of mercury gives a yellow precipitate with solution of sodium carbonate, which is used as an *indicator* in a manner to be described.

The urea solution to be analyzed is poured into a beaker and standard solution of the mercuric nitrate added gradually, with constant stirring, from a burette. From time to time a drop of the liquid above the precipitate is taken on the end of a glass rod and brought in contact with a few drops of a concentrated solution of sodium carbonate on a plate of dark glass. A yellowish precipitate forms here if the drop contains any

excess of the mercury compound beyond that necessary to precipitate the urea. The end of the reaction is frequently determined in this manner, as in the original process, but not with greatest accuracy. A modified process, as now to be explained, is preferable, and easily carried out.

As the equation above shows, nitric acid is set free in the reaction between the urea and the mercuric nitrate. This acid has a decomposing effect on the precipitate, tending to form new nitrate and thus diminish the amount which, theoretically, should be added for complete precipitation. The nitric acid must therefore be neutralized from time to time as formed, or better, just before the end reaction with the indicator is tried.

It has been found, also, that to precipitate exactly 10 milligrams of urea in this manner, not 72 milligrams of mercuric oxide in solution, but a slightly greater amount must be used. The experiments of Pflueger showed that 77.2 milligrams is needed for the purpose and the standard solution should be made to contain 77.2 grams per liter.

In the titration of urine certain modifications must be made which are not necessary in the titration of pure urea solutions. The phosphates, sulphates, and chlorides of urine interfere with the reaction and must be removed before the test is begun.

The phosphates and sulphates may be removed by precipitation with barium solution, while the chlorides may be thrown out by silver nitrate. It is also possi-

ble to make a correction for the chlorides instead of precipitating them. The following solutions are necessary in making the test:

(*a*) **Mercuric Nitrate Solution.**—This is made of definite strength and should contain the equivalent of 77.2 grams of the oxide in one liter. In making this solution we may start with pure metallic mercury, with mercuric oxide or with the commercial nitrate (mercurous). With mercury it can be made in this manner: Weigh out a quantity of pure mercury and heat it in a porcelain dish or casserole with two or three times its weight of strong nitric acid of 1.42 specific gravity. When the mercury is in solution evaporate to the consistency of a thick sirup and add from time to time a few drops of nitric acid to complete the oxidation. When the addition of the acid is no longer followed by the evolution of red fumes the action is complete and the mercury exists as mercuric salt. Now pour into the sirupy residue ten times its volume of water, with constant stirring. In adding the water it always happens that a little of the nitrate is decomposed and thrown down as a basic salt. Allow the liquid to settle thoroughly, pour off from the sediment and dissolve the latter in a few drops of nitric acid. Add this now to the main solution and dilute it with distilled water to make 1 liter of each 71.5 grams of mercury.

When mercuric oxide is employed, weigh out the proper amount, dissolve it in a slight excess of strong, pure nitric acid, evaporate to a sirup and treat as above. Finally, dilute with water to yield a solution with 77.2 grams to the liter.

(*b*) **Baryta Solution** to precipitate phosphates and sulphates. To one volume of a cold saturated solution of barium nitrate add two volumes of a cold saturated solution of barium hydroxide. Keep in a well-stoppered bottle.

(*c*) **Sodium Carbonate Solution.**—This is best made of the pure, dry carbonate readily obtained as a commercial article. It must be remembered, however, that the so-called dry carbonate contains a little water, which may be removed by heating, in a platinum dish, to low redness. Dissolve 53 grams of the salt thus dried, in water, and dilute to one liter.

A mercury solution made of pure material according to the above directions should have the correct strength, but for control it may be tested by means of a solution of pure urea in water.

(*d*) **Standard Urea Solution.**—Weigh out 2 grams of pure urea, which is now readily obtained, and dissolve it in distilled water to make 100 cc.

Before making the test proper it is necessary to determine the relation of the sodium carbonate solution to the mercury solution in presence of urea. This may be done by taking exactly 10 cc. of the urea solution and adding to it 19 cc. of the solution of mercuric nitrate. Shake thoroughly, allow to stand a minute and filter. Wash the precipitate with a little distilled water, and to the mixed filtrate and washings add two drops of a weak solution of methyl orange, or enough to give a pink color. Then from a burette

run in the solution of sodium carbonate, with constant shaking, until the pink color changes to yellow. The reaction is sharp enough for the purpose. Not over 11.5 cc. of the alkali solution should be required for this. Calculate the amount needed for each cubic centimeter of mercuric nitrate. Proceed now with the actual test.

Measure 10 cc. of the standard urea solution again and run in 19.5 cc. of the mercuric nitrate. Add now the correct number of cubic centimeters of soda solution required to neutralize the acid from the nitrate, as calculated from the results of the last experiment. Then by means of a stirring rod bring a drop of the liquid in the beaker in contact with a drop of a semifluid mixture of sodium bicarbonate and water on a dark glass plate. Stir the two together and observe the color. It should be white. Run in two or three drops more of the mercury solution, stir well and repeat the test, and continue until a slight yellow color is obtained on mixing the drops from the beaker with the moist bicarbonate. If the mercury solution is correct, just 20 cc. should be used for this.

The sodium bicarbonate used as indicator must be pure, especially as regards freedom from chloride. An excess of it can be washed in a beaker several times with a little cold water, which is poured off leaving the salt finally in a pasty condition suitable for use.

We proceed now to the actual test of a sample of urine. Measure off accurately a definite volume and add to it just half its volume of the baryta solution. Convenient proportions are 50 cc. of urine and 25 cc.

of the baryta solution. Shake thoroughly and filter through a dry filter into a flask. Measure out now exactly 15 cc. of this filtrate which contains the urea of 10 cc. of the original urine, the baryta solution having taken out only phosphates, sulphates, and carbonates, with certain bases. This filtrate still contains chlorides which are objectionable and which could be separated by another precipitation with the proper amount of silver nitrate. It will be, however, more convenient, and fully as accurate, to make a correction for them at the end of the test, as will be explained. The 15 cc. of filtrate has an alkaline reaction and must be neutralized. (If not alkaline a new precipitation must be made, taking equal volumes of urine and baryta solution, and finally 20 cc. of the filtrate.) The neutralization can be effected by adding carefully, a drop at a time, dilute nitric acid, testing with litmus paper.

The filtrate thus prepared is titrated with the mercury solution. Begin by adding a cubic centimeter at a time and after each addition bring a drop of the mixture in contact with a drop of the semifluid sodium bicarbonate on a plate of dark glass. The drops should be placed side by side and mixed at the edges. At first the mixture remains white, even after stirring, but as the addition of mercury is continued a point is reached where the drop from the beaker brought in contact with the moist bicarbonate gives a light yellow shade. On stirring the drops together this yellow should disappear, but this shows that the end of the reaction is nearly reached. Add now the mercury

solution in drops and test after each addition. When the point is reached where a faint yellow shade persists after stirring together the drop from the beaker and the sodium bicarbonate, it is time to neutralize with the normal sodium carbonate solution. Run in the right number of cubic centimeters corresponding to the mercury used and now make the test for the final reaction again and continue until the yellow color appears.

Regard this test as preliminary and make a new one with 15 cc. of the filtrate neutralized as before. Run in directly within 1 cc. of the amount of mercury required, as shown by the first test, neutralize and complete as before. For each cubic centimeter used, *after deducting for chlorides*, calculate 10 milligrams of urea. The correction for the chlorides is based on the following principle: In the presence of the sodium chloride, or other chloride, the reaction between mercuric nitrate and urea does not begin until enough of the former has been added to form mercuric chloride with all chlorine present, according to the following equation:

$$Hg(NO_3)_2 + 2NaCl = 2NaNO_3 + HgCl_2.$$

For the nitrate corresponding to 216 parts of HgO we use here 117 parts of salt, or for 117 milligrams of salt, 216 milligrams of mercuric oxide, or 2.79 cc. of the standard mercuric nitrate solution.

One milligram of sodium chloride, therefore, combines with the mercury compound in 0.0238 cc. of the standard solution. Mercuric chloride does not react

with the sodium bicarbonate, and the amount formed is beyond that shown by the titration. Therefore, to apply the correction, determine the chlorides present in 10 cc. of urine (by a process to be given later), calculate to sodium chloride, and for each milligram found deduct 0.0238 cc. from the volume of the mercuric nitrate solution used in the titration. As the amount of chlorine in the urine is about equivalent to 1 per cent. of salt, in the mean, an approximate correction is often made by subtracting 2 cc. from the volume of the mercury solution.

The above calculations are based on the supposition that the original urine contains 2 per cent. of urea, and that a volume of about 20 cc. of mercuric nitrate is used in the titration. If the per cent. of urea is much greater or less than this, a correction on account of volume must be made. This correction has been worked out empirically by Pflueger, and, without discussing how it is derived, it will be sufficient to explain its application.

If more than 2 per cent. of urea is present it is necessary to add more than 20 cc. of the mercuric solution in titration. If the volume of the latter solution is greater than the sum of the volumes of the prepared urine and soda solution used in neutralization, this sum must be subtracted from the volume of the mercury solution and the result multiplied by 0.08. The product is added to the number of cubic centimeters of mercuric nitrate used, to give the corrected result. If, on the other hand, the volume of mercuric nitrate used in titration is less than the sum of the volumes

of prepared urine and soda solution, the difference is multiplied by 0.08 and the product taken from the number of cubic centimeters of mercuric solution used, to give the corrected result. In these calculations the volume of mercuric nitrate taken up by the chlorides must be considered as part of the diluting liquid. The same must be remembered in adding sodium carbonate for neutralization.

The correction may be expressed in this formula, according to Pflueger:

$$C = (V_1 - V_2) \times 0.08,$$

in which

C = the correction to be added or subtracted;
$V_1$ = the sum of the volumes of the urine, soda solution and mercuric nitrate combined with the chlorides;
$V_2$ = the volume of mercuric nitrate taken by urea.

In illustration we may take an actual case:

15.0 cc. = the prepared urine (neutralized);
15.8 cc. = the sodium carbonate used;
1.8 cc. = the mercuric solution used by chlorides.

$V_1$ = 32.6 cc.
$V_2$ = 26.0 cc.

6.6

$$(V_1 - V_2) \times 0.08 = 0.528 = C.$$

Therefore, 26 — 0.5 = 25.5 is the corrected volume of mercuric nitrate, indicating, with the latter solution of the standard strength, 25.5 grams of urea in a liter.

The results of the Liebig method are usually a little high because urine contains, besides urea, several other substances which react with the mercuric nitrate. The first of these is ammonia, which is generally present in small amount in normal fresh urine and often in larger amount in pathological urines. A process for the determination of the ammonia will be given later. It has been found that 10 milligrams of $NH_3$ require about 2.0 cc. of the standard mercuric solution and on this basis a correction may be made. In normal urine the correction would amount to about 1.0 cc. of the mercuric solution, in the mean, for the volume of filtrate used.

Uric acid, hippuric acid, creatin, creatinin and traces of other bodies containing nitrogen, have also a disturbing action and make the calculated per cent. of urea appear higher than it should be. A slight correction should be therefore introduced here too. Taking all the nitrogenous bodies, including ammonia, into consideration, it is safe to subtract 2 cc. from the volume of the mercuric solution used to find that actually required by the urea.

If the urine contains albumin this must be coagulated and filtered out. To accomplish this, measure 50 cc. of the urine, add a few drops of acetic acid and boil to completely coagulate. Allow to cool, filter and add a little water to compensate for that lost by evaporation in the boiling. With the filtrate proceed as before.

From what has been said it will be recognized that the Liebig process is one for the determination of the

total nitrogenous matter in urine rather than for the titration of urea alone. Because of the several corrections which must be applied to the results as obtained, many chemists prefer to employ a different process, depending on an entirely different principle, which will now be explained.

**Method by Liberation of Nitrogen.** — A solution of urea is decomposed by a solution of a hypochlorite or hypobromite as illustrated by this equation.

$$CON_2H_4 + 3NaOCl = CO_2 + N_2 + 2H_2O + 3NaCl.$$

That is, nitrogen and carbon dioxide gases are given off. If the reaction is allowed to take place in an alkaline medium the carbon dioxide will be held and the nitrogen alone given off. The volume liberated is a measure of the weight of urea decomposed. From the above equation it is seen that 28 parts by weight of nitrogen correspond to 60 of urea, from which it follows that 1 cc. of pure nitrogen gas, measured at a temperature of 0° C. and under the normal pressure of 760 mm. corresponds to 0.00269 gram of urea. One gram of urea furnishes 371.4 cc. of nitrogen gas.

In employing these principles in practice it is simply necessary to bring together a measured volume of the urine or urea solution and the hypochlorite or hypobromite reagent under such conditions that all of the nitrogen liberated may be collected and accurately measured.

As a reagent a solution of sodium hypobromite is very commonly employed. As it does not keep well it must be made fresh for use, which is inconvenient

unless many tests have to be made at one time. The reagent may be prepared in this manner:

Dissolve 100 grams of good sodium hydroxide in 250 cc. of water. When cold add 25 cc. of bromine by means of a funnel tube carried to the center of the solution. The bromine must be poured into the funnel, a little at a time, and the lower end moved around to act as a stirrer and mix the liquids. During the reaction the bottle or flask containing the alkali should be surrounded by cold water, and the mixture should be made out of doors or in a good fume closet. The finished solution contains an excess of alkali sufficient to hold the carbon dioxide given off when it reacts on urea. In place of this solution a strong hypochlorite solution may be used with advantage. The U. S. P. solution *when properly made* answers very well. Its preparation is given in the appendix.

Many forms of apparatus have been devised for this purpose, one of the oldest and best known of which is that of Huefner, shown in Fig. 3.

At *A*, below, is a small vessel, the bottom of which rests on the support, and which holds the urine to be decomposed. The capacity of this vessel is usually between 5 and 10 cc., but must be accurately determined by filling with mercury, pouring this out and weighing it. Above *A*, and separated from it by a ground glass stop-cock, is the much larger vessel *B*, which holds the hypochlorite or hypobromite reagent. On the narrow neck of *B* rests a cup-shaped receptacle, *C*, to hold water. Finally, over *B* a measuring tube is supported in such a manner that it must re-

ceive any gas passing up from *B*. At the beginning of the experiment this measuring tube *D* is filled with water.

The apparatus is used in the following manner: Rinse out *A* and *B* and pour into the latter more than enough urine to fill *A*. Open the stop-cock and allow the urine to flow down, which may be assisted, when slow, by moving a thin glass rod or bit of wire up and down through the opening in the stopper. When *A* is quite full, close the stopper, rinse the urine from *B* and fill it with the hypobromite reagent. Now fill the cup *C* with water so that its surface is one or two centimeters above the opening into *B*, fill the graduated tube with water, close the end with the thumb and invert it in the cup. Finally fasten it in position over *B* as shown.

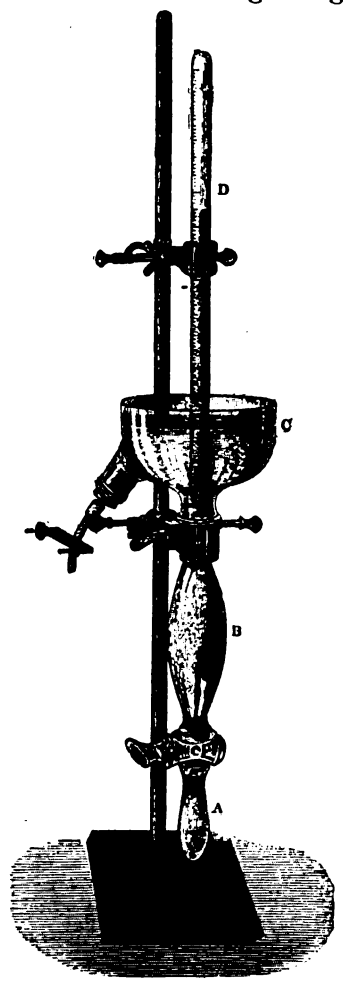

Fig. 3.

The stop-cock is now opened which permits the

heavier reagent to sink and slowly mix with the urine. A liberation of gas soon begins and proceeds slowly. At the end of twenty or thirty minutes the reaction is complete, which can be noted by the disappearance of the gas bubbles. Close the end of the gas tube with the thumb, remove it and immerse in a jar of water, having the temperature of the air, until the water levels inside the tube and in the jar, are the same. Clamp the tube in position and allow it to stand until the temperature of the gas becomes constant, which may require fifteen minutes. Finally adjust the tube level again, if necessary, read the volume of the gas, note the height of the barometer and the temperature, as given by a thermometer hanging near the tube, and reduce this volume to standard conditions by the following formula:

$$V_c = V_c \cdot \frac{b - w}{(1 + 0.00366t)\,760}$$

In this formula

$V_c$ = the corrected volume;
$V_o$ = the observed volume;
b = the barometric height;
w = the tension of water vapor at the observed temperature, in millimeters of mercury;
t = the temperature as observed.

The values for w at different temperatures are given in this table:

| t | w | t | w | t | w |
|---|---|---|---|---|---|
| 10° | 9.165 mm. | 16° | 13.536 mm. | 21° | 18.495 mm. |
| 11° | 9.792 mm. | 17° | 14.421 mm. | 22° | 19.659 mm. |
| 12° | 10.457 mm. | 18° | 15.357 mm. | 23° | 20.888 mm. |
| 13° | 11.162 mm. | 19° | 16.346 mm. | 24° | 22.184 mm. |
| 14° | 11.908 mm. | 20° | 17.391 mm. | 25° | 23.550 mm. |
| 15° | 12.699 mm. | | | | |

Having thus the volume of the gas under normal conditions, the weight of urea corresponding can be calculated by data already given.

The results obtained by this method are too low and must be corrected, as will be explained later.

Another form of urea apparatus which can be constructed by any one is shown in Fig. 4.

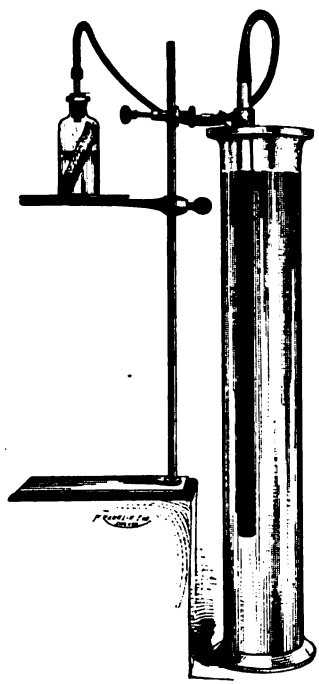

Fig. 4.

The tall jar is filled with water which must stand until it has the air temperature. A 50 cc. burette is inverted in the jar, the delivery end being connected with a bottle holding about 150 cc., by means of a piece of firm rubber tubing. The rubber tube is slipped over a short glass tube passing through the hole in a rubber stopper which must close the bottle accurately. In the bottle is a short stout test-tube, or vial, holding about 10 cc. and which contains the urine to be tested. Into the bottle itself is poured the reagent as above described, which must not reach to the top of the test-tube. On mixing the liquids the urea decomposes, liberating the gas as

before, which passes through the rubber tube and displaces water in the burette so that the volume can be readily determined.

The test is made practically in this manner: Pour about 20 cc. of the strong hypobromite or 40 cc. of the weaker hypochlorite reagent into the bottle. With a pipette measure some exact volume of urine, usually 5 cc., into the small test-tube, and by means of small iron forceps place the latter carefully in the bottle containing the reagent. Next insert the stopper which connects the bottle with the burette standing in the jar of water. The level of the water in the burette is displaced by this operation. Now allow the apparatus to remain at rest ten minutes until the air in the mixing bottle and tube has the temperature of the outside air. Through handling, it of course becomes warmer. At the end of the time, by means of the attached clamp, and not by the hand, lift the burette, or depress it, if necessary, until the water levels inside and outside are the same. Note the position of the water on the burette graduation. Read the air temperature by a thermometer, which should be suspended near the tube, and observe the height of the barometer.

Incline the bottle to mix the urine and the reagent, shake gently and repeat these operations from time to time. On the decomposition of the urea nitrogen gas, or its equivalent volume of air, passes over into the burette and forces out some of the water. When, after repeatedly shaking the bottle, no increase of the gas volume in the burette can be observed, allow the whole apparatus to stand until the contents of the bottle and

burette have cooled down to the air temperature again. Then lift the burette, with the clamp as before, to restore the levels and read the gas volume. From this subtract the volume at the first reading. The difference is the volume of nitrogen gas liberated in the reaction, at the observed temperature and atmospheric pressure. If, as sometimes happens, more gas is liberated than the burette will hold, repeat the experiment, using urine diluted with an equal volume of water.

The calculations are made as in the case given above, as the gas volume is finally measured under the same conditions. It is assumed here that the air temperature and barometric pressure remain constant during the experiment.

Exact investigations have shown that the whole of the nitrogen is not liberated in the reaction, as at first assumed, but falls short between 7 and 8 per cent. It appears that under some conditions a small part, possibly 3 or 4 per cent., of the nitrogen of the urea is oxidized to nitric acid in the reaction and escapes measurement. Another small portion is left in the ammoniacal condition. Attempts have been made to prevent this abnormal oxidation by adding to the urine a reducing agent to destroy nitric acid as fast as formed. Dextrose has been used for the purpose, also cane-sugar, and apparently with success. But a great excess of sugar must be added.

It has been shown, also, that the theoretical yield of nitrogen can be approached by a modified method of applying the reagent. J. R. Duggan, working in

Remsen's laboratory, found that by mixing the alkali with the urine and then adding the bromine so as to form hypobromite in presence of the urea, the yield of nitrogen is much increased, reaching nearly the amount called for by theory. The simple bottle and burette apparatus may be used in this manner by measuring the alkali, 20 cc. of 20 per cent. sodium hydroxide solution, and urine, 5 cc., into the bottle, and the bromine, about 1 cc., into the test-tube. The mixture is made and process completed as before. The modification has not come into general use, probably because of the objection to working with free bromine, at each test. As the deficiency by the usual method has been shown to be nearly 8 per cent., it is sufficient for most purposes to assume that the volume of gas obtained is 92 per cent. of the whole and correct by calculation.

It should also be stated here that there are certain positive errors in the process as in that of Liebig. The hypobromite acts not only on urea, but on uric acid, ammonia and on other normal urine constituents. In most of these cases not all of the nitrogen present is given off in the free state, but the fraction which is liberated is enough to cause a very sensible error in the process. It is generally overlooked because the opposite or minus error is so much greater. Attempts are sometimes made to remove these disturbing substances by precipitation with phosphotungstic acid before applying the Liebig or hypobromite process, but in every-day clinical practice this complication cannot be recommended.

It remains now to describe two forms of apparatus which are used without any correction, for rapid clinical tests.

**The Squibb Apparatus** is the first of these, and one which can be very highly recommended. The construction of the apparatus is shown by Fig. 5.

The upright bottle to the left contains the reagent, hypochlorite or hypobromite as before. By means of a pair of small forceps a short test-tube, *F*, containing a measured volume of the urine is dropped into the bottle, but carefully, so that the liquids will not mix

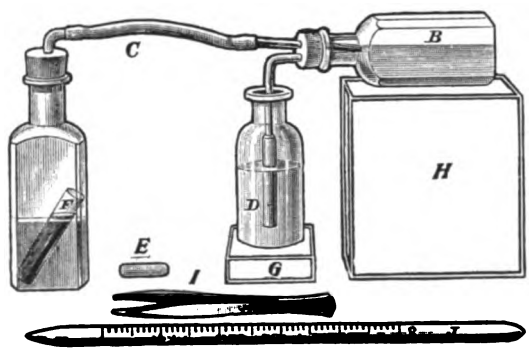


Fig. 5.

until the bottle is shaken. A bent glass tube and a rubber tube connect this with the bottle *B*, which at the beginning of the test is quite filled with water. Another glass tube connects *B* with the bottle *D*, empty at the beginning of the test.

To make the test pour into the first bottle 20 cc. of strong hypobromite solution, or 40 cc. of the hypochlorite. Measure accurately 4 or 5 cc. of urine into

the tube, drop this into the bottle and insert the stopper. Fill *B* quite full of water, and insert its stopper which drives out a little water through the short tube into *D*. Allow the whole apparatus to stand ten minutes to take the temperature of the air, then empty *D* and replace it. Now tip the first bottle so as to mix the contents of *F* with the reagent and shake gently. Bubbles of gas escape, and, passing over into *B*, drive out a corresponding volume of water. Repeat the shaking of the reagent bottle several times. In a few minutes the reaction is complete, but the apparatus must be allowed to stand to cool down to the air temperature. A part of the water in *D* may be drawn back into *B*. Finally measure the volume of water left in *D*, and take this as the volume of gas liberated. Make the calculation as before on the assumption that each cubic centimeter of gas corresponds to 0.0027 gram of urea. In this there are two errors which nearly compensate each other. In the first place not all the gas is liberated for the reasons explained above, but in the second place the volume read off is higher than normal because of higher temperature and lower barometer. The results obtained may be, therefore, nearly correct, sufficiently so for all clinical purposes, through compensation of errors.

Squibb has constructed a table from which the percentage of urea corresponding to any volume of gas, for a given volume of urine taken, can be read at a glance without any calculation whatever. This is convenient, but not necessary.

**The Doremus Apparatus.**—This is shown in the annexed cut, and at the present time is very widely used by physicians because of the simplicity of the method of employing it.

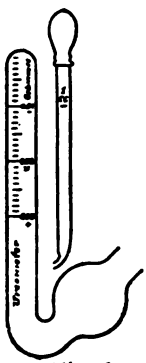

Fig. 6.

The graduated tube is filled to an indicating mark with strong hypobromite solution and then water is added to fill the remainder of the tube and the bulb. By means of the pipette, graduated to hold 1 cc., this quantity of urine is forced into the liquid in the upright part of the tube. The urea is immediately decomposed, and its nitrogen ascends to the top of the graduated part, where it is read off. The longer marks on the tube indicate the fraction of a gram of urea in the 1 cc. of urine taken; the shorter marks indicate tenths. Multiplying by 100 we obtain the number of grams of urea in 100 cc. The results are apt to be low from a loss of nitrogen through the bulb, which can scarcely be avoided. The instrument is said to be "good enough" for clinical purposes, but cannot be compared with that of Squibb for accuracy. No instrument in which the volume of urine taken for the test is as small as 1 cc. should be expected to give even approximately accurate results, unless very great precautions are taken in the measurement and in subsequent parts of the work.

**The Total Nitrogen.**—By this is understood the nitrogen of all the excreted urinary products. It is approximately measured by the Liebig process, uncorrected,

and may be determined with perfect accuracy by several methods. The Kjeldahl process is most conveniently applied but, like all the others, in it too many manipulations are required to make it available for clinical purposes. For a description of the details of the Kjeldahl process the reader is referred to special works on organic analysis.

# CHAPTER VII

## THE DETERMINATION OF PHOSPHATES, CHLORIDES, AND SULPHATES

### Phosphates

Phosphoric acid occurs normally in the urine combined with alkali and alkali-earth metals, of which combinations the alkali phosphates are soluble in water, while the earthy phosphates are insoluble. In the urine, however, they are held in solution through several agencies. The larger part of the earthy phosphates appear to be held here normally in the acid condition; that is, as compounds of the formulas $CaH_4(PO_4)_2$ and $MgH_4(PO_4)_2$. The salts of the type $CaHPO_4$ are present, also, in small amount. As long as the urine maintains its acid reaction these bodies may be expected to remain in solution, but if it becomes alkaline by fermentation, or by the addition of the hydroxides or carbonates of ammonium, sodium, or potassium, the acid phosphates are converted into insoluble, neutral phosphates and precipitated. Most urines contain along with the acid phosphates traces of neutral phosphates which precipitate on boiling. It has been suggested that these phosphates are held by traces of ammonium compounds or by carbonic acid, both of which are driven off by heat, allowing the phosphates to precipitate. It is well known, how-

ever, that some urines can be boiled without showing any sign of precipitation. In such cases it is probable that the neutral phosphates are not present.

Part of the phosphoric acid of the urine comes directly from the phosphates of the food and another portion results from the oxidation of the phosphorus-holding tissues. In health, the rate of such oxidation is practically constant, or nearly so, but in disease it may be greatly increased or diminished. Variations in the amount of excreted phosphates may become, therefore, of considerable clinical importance. Great care must be observed, however, in drawing conclusions regarding the destruction of phosphatic tissues from the results obtained by analysis, because of the uncertainty of the amount taken with the food and of what must be considered a normal excretion of phosphoric acid.

Wheat bread constitutes one of our important articles of food containing a relatively high amount of phosphates. Owing to changes in milling processes introduced and extended in the past twenty years, very material reductions have been made in the percentage of phosphates and other mineral substances left in our fine flour. The resulting diminution in the phosphates of the urine from this cause is appreciable. It must be remembered also that a part of the phosphoric acid taken with the food is eliminated in insoluble form with the feces. The amount so disposed of depends on the nature of the original condition of combination of the phosphoric acid and on the amount of alkali-earth bases present. These tend to form insoluble

phosphates. With an exclusively vegetable diet the phosphates would, for this reason, be low, while with a meat diet more would be excreted, because here the alkali phosphates, especially potassium phosphate, are in excess. On the other hand the phosphates are greatly increased in the urine of people who consume great quantities of the various phosphatic beverages which have become popular in the United States in the past few years. It is readily seen how quite erroneous conclusions could be drawn from the tests of urine of such persons. Single analyses for phosphoric acid may be very misleading. When the amount of phosphoric acid passed with a given diet is known, variations observed may sometimes be traced to certain pathological conditions briefly mentioned in the following paragraph.

In disease, phosphates are found increased in *rickets* and *osteomalacia*, possibly from the failure to deposit the earthy phosphates in the bones. Meningitis is accompanied by an increase of the phosphates. The same is true of other disorders of the brain as this organ is especially rich in phosphatic substances. It is said that phosphates are increased after a period of severe nervous strain. What has been termed *phosphatic diabetes* has been described as a condition in which a persistent excretion of relatively large amounts of the phosphates is observed. The earthy phosphates so discharged may amount to 20 grams or more in a day. The causes leading to this condition are not clearly defined.

The phosphates have been found diminished in diseases accompanied by diminished nutrition, and in some diseases of the heart and kidney. In dilute urine, low specific gravity and high volume, the *percentage* amount of phosphoric acid is much decreased, but it does not follow that a decrease in the amount excreted in the twenty-four hours must also be small. A safe estimate can be made only with a mixed specimen taken from the urine of the whole day with consideration of the volume passed.

Various statements are found in the books regarding the mean excretion of the alkali and earthy phosphates. Different observers have reported between 2 and 5 grams of phosphoric anhydride ($P_2O_5$), while 3 grams may be taken, perhaps, as the mean.

The recognition of the phosphates is an extremely easy matter. The presence of earthy phosphates may be shown by adding to the urine enough ammonia water to give a faint alkaline reaction and then warming. A flocculent precipitate, resembling albumin, appears and is usually white, or nearly so. But sometimes coloring-matters come down with it in amount sufficient to give it a brownish or reddish shade. It will be recalled that the color of this precipitate was referred to under the head of blood tests.

The alkali phosphates can be detected in the filtrate after separation of the earthy phosphates. To this end, add to the clear alkaline liquid a little more ammonia and some clear magnesia mixture. A fine crystalline precipitate of ammonium-magnesium phosphate separates and settles rapidly. This is very character-

istic. The qualitative tests for phosphates have, however, little value in examination of the urine. We are chiefly concerned with the amount, the measurement of which will now be described.

**Determination of Phosphates.**—It is customary to measure the total phosphoric acid, not the alkali or earthy phosphates, separately. We have at our disposal several methods, gravimetric and volumetric, of which the latter are accurate and most convenient. A volumetric process will be described which serves for the measurement of the phosphoric acid as a whole, and which can be used for the separate measurement of the earthy and alkali phosphates by dealing with the precipitate and filtrate described in the qualitative test above. This method depends on the fact that solutions of uranium nitrate or acetate precipitate phosphates in greenish-yellow colored, flocculent form, and that in a solution holding in suspension a precipitate of uranium phosphate any excess of soluble uranium compound may be recognized by the reddish brown precipitate which it gives with a solution of potassium ferrocyanide. The latter substance serves, therefore, as an indicator. If to a phosphate solution in a beaker a dilute uranium solution be added precipitation continues until the whole of the phosphates have gone into combination with the uranium. If, during the precipitation, drops of liquid from the beaker are brought in contact with drops of fresh ferrocyanide solution on a glass plate, no reddish brown precipitate of uranium ferrocyanide appears until the last trace of uranium phosphate has been formed. The produc-

tion of uranium ferrocyanide is the indication, therefore, of the finished precipitation of the phosphates.

The reaction between uranium and phosphates in acetic acid solution is illustrated by this equation:

$$UO_2(NO_3)_2 + KH_2PO_4 = UO_2HPO_4 + KNO_3 + HNO_3$$

From this it appears that 239.6 parts of uranium are required to precipitate 71 parts of $P_2O_5$. In order to have the reaction take place as represented above, it is necessary to neutralize the liberated nitric acid as fast as formed or dispose of it in some other manner. The best plan is to add to the solution to be precipitated acetic acid and *sodium acetate*, the first of which brings the phosphates into the acid condition, while the second is decomposed with the formation of sodium nitrate and free acetic acid. Mineral acids interfere with the reaction, while moderate amounts of acetic acid do not.

In order to carry out this method we prepare the following solutions:

(*a*) **Standard Uranium Solution.**—This is made by dissolving 36 grams of the pure crystallized nitrate, $UO_2(NO_3)_2 6H_2O$, in distilled water to make one liter. The strength of the solution is adjusted by experiment as explained below.

(*b*) **Standard Phosphate Solution.**—This is made by dissolving 10.085 grams of pure crystals of sodium phosphate, $HNa_2PO_4 . 12H_2O$, in distilled water to make 1 liter. 50 cc. of this solution contains 0.100 gram of $P_2O_5$. Small fresh, uneffloresced crystals of the phosphate must be used for this solution.

(*c*) **Sodium Acetate Solution.** — Dissolve 100 grams in 800 cc. of distilled water, add 100 cc. of 30 per cent. acetic acid and then water enough to make 1 liter.

(*d*) **Fresh Ferrocyanide Solution.**—Dissolve 10 grams of pure potassium ferrocyanide in 100 cc. of distilled water. The solution should be kept in the dark.

The actual value of the uranium solution is determined by the following experiment: Measure out 50 cc. of the phosphate solution, (*b*), add 5 cc. of the acetate solution, (*c*), and heat in a beaker in a water-bath to near the boiling temperature. Place several drops of the ferrocyanide solution on a white plate. Fill a burette with the uranium solution and when the solution in the beaker has reached the proper temperature run into it from the burette 18 cc. of the uranium standard. Warm again, and by means of a glass rod bring a drop of the liquid in the beaker in contact with one of the ferrocyanide drops on the plate. If the uranium solution has been properly made no red color should yet appear. Now run in a fifth of 1 cc. more from the burette, warm and test again, and repeat these operations until the first faint reddish shade begins to show on bringing the two drops in contact. With this test as a preliminary one make a second, adding at first one-fifth of a cubic centimeter less than the final result of the preliminary, and finish as before. Something less than 20 cc. should be needed to complete the reaction. Supposing 19.8 cc. are required for the purpose, the whole solution should be diluted in the proportion,

$$19.8 : 20 :: a : x$$

in which *a* represents the volume on hand. We have now a standard uranium solution, each cubic centimeter of which precipitates exactly 5 milligrams of $P_2O_5$, and with this we are able to measure phosphoric acid in *unknown* solutions.

It is sometimes recommended to use uranium acetate instead of nitrate in making this standard solution, and so avoid a disturbing element in the liberation of the nitric acid. But there are several advantages in the use of the nitrate which should be mentioned. In the first place its solutions keep better, and secondly, it can be obtained in commerce in almost (if not quite) chemically pure condition, so that it is possible to make up a solution of nearly correct strength by simply weighing out and dissolving the crystals. Assuming 239.6 as the atomic weight of uranium, and O = 16, the relation of the crystallized pure nitrate to $P_2O_5$ is

1007.2 : 142,

from which it follows that a liter of the standard solution should contain 35.46 grams of the uranium salt, if each cubic centimeter is to indicate 5 milligrams of $P_2O_5$.

The solution of sodium acetate with acetic acid must always be added in the proportion given above if uniform results are expected, and the ferrocyanide indicator must be fresh and as weak as given.

The test of urine is made exactly as given in the above. Measure out 50 cc. of urine, add 5 cc. of the acetate mixture, and finish as before. The 50 cc. of urine, in the mean, contains about as much phosphoric

acid as was present in the same volume of standard phosphate solution. The titration must be made hot, because the reaction is much quicker and sharper in hot solution than in cold. Make always two tests; the first is an approximation, while the second gives a much closer result.

A separate test of the earthy phosphates may be made by adding to 200 cc. of urine enough ammonia to give an alkaline reaction. The urine then must stand until the precipitated phosphates settle out. The precipitate is collected on a small filter, washed with water containing a very little ammonia, and then allowed to drain. It is next dissolved in a small amount of acetic acid, the solution diluted to 50 cc., mixed with 5 cc. of the sodium acetate solution and titrated as before. The reaction here is not quite as accurate as with the alkali phosphate, but the results are satisfactory for the purpose. The difference between the total phosphates and the earthy phosphates, expressed in terms of $P_2O_5$, is the amount combined as alkali phosphates.

It is also possible to determine the amount of phosphoric acid combined as monohydrogen salt and that combined as dihydrogen salt, but the determination has at the present time little clinical value.

Instead of finding the end point in the precipitation with uranium solution by means of drops of ferrocyanide as explained, the following process may be followed. Add to the urine the sodium acetate as before and then three or four drops of cochineal solution, made as given in the appendix. Heat to boiling and

add the uranium solution to the hot liquid. Just as soon as the phosphate is combined and a trace of uranium left in excess, it produces a green color or precipitate with the cochineal which thus serves as an *indicator* to show the end of the reaction. If the urine is quite warm the color is sharp and can be quickly seen so that the reaction may be considered a sensitive one. The same test may, of course, be used to standardize the uranium solution at the outset.

### Chlorides

Practically, all of the chlorine taken into the stomach with the food is eliminated with the urine. The chlorine entering the body is mostly in the form of sodium chloride, although traces of other chlorides are found in some of our foodstuffs.

The amount of salt excreted depends, therefore, closely on that consumed, and varies within wide limits. It is commonly said that 10 to 15 grams daily include the amounts passed in the great majority of urines. It must be remembered, however, that in individual cases the upper limit may be very greatly exceeded. In the urine of men who eat a great deal of salt food, from 20 to 25 grams are frequently found. In such cases the volume of water drunk is usually large, so that the *percentage* amount of salt passed does not follow the same variations. Expressed in this manner, about 1 per cent. represents the average excretion.

Pathologically, chlorides are increased in total amount in *diabetes insipidus*, and temporarily, some-

times in intermittent fevers. A decrease in the chlorides is more frequently observed, and has greater clinical importance.

This decrease is noticed generally in febrile conditions, and especially if salty exudations are being formed in any part of the body. In the serous accumulations of pleurisy common salt is abundant, and at the same time greatly diminished in the urine. The expectorated fluid in cases of acute pneumonia contains much salt, which in consequence is decreased in the urine. In some cases the chlorine is practically absent from the urine. In any event its reappearance in normal amount during the progress of disease is a favorable sign, as indicating the approach of normal conditions of absorption and excretion in the body. Quantitative tests for the chlorides of the urine become, therefore, of great importance, as their absence or marked decrease can only occur in disorders of serious nature. Fortunately, such tests are very easily made, and by simple volumetric processes.

**Volumetric Determination of Chlorine.**—The best processes by which chlorine is measured volumetrically in the urine and elsewhere depend on the reaction between chlorides and silver nitrate,

$$NaCl + AgNO_3 = AgCl + NaNO_3,$$

from which it appears that 5.85 milligrams of salt require for precipitation 16.99 milligrams of silver nitrate. In many cases, if a weak solution of silver nitrate be added to a weak solution of salt in a bottle, with frequent shaking, the curdy precipitate of silver

chloride formed will settle out so rapidly that it is possible to determine just when the reaction is complete, from the formation of no further precipitate in the nearly clear liquid, as drops of silver nitrate mix with it. A chloride can be added to precipitate silver from a solution of its nitrate in the same manner, and so delicate is the reaction that a method based on it is still employed in many mints for determining the amount of silver in bullion, coin, or other alloy.

For the determination of chlorides in solutions, especially in urine, other methods, more convenient and fully as accurate, are employed. If a weak solution of silver nitrate be added to a neutral solution of a chloride containing enough potassium chromate to impart a slight yellow color to it the silver combines with the chlorine first and then, only after this has been completely precipitated, with the chromic acid to form brick-red silver chromate. In such a mixture the appearance of the first tinge of red is the indication that the chlorine has been wholly precipitated. The neutral chromate is used here as the indicator. As each drop of silver nitrate solution falls into the solution of chloride and chromate, a transitory reddish color may appear before the reaction is completed, but this vanishes on shaking or stirring the liquid and becomes permanent only when the chlorides are completely combined with silver. In using this method the following solutions are employed:

(*a*) **Standard Silver Nitrate Solution,** N/10. — Dissolve 16.99 grams of pure fused silver nitrate in distilled water and dilute to 1 liter. Silver nitrate can

usually be obtained of sufficient purity for the purpose, from dealers in fine chemicals, but should be fused in a porcelain crucible at a low temperature before being weighed out. The correctness of the solution may be tested by the following:

(*b*) **Standard Sodium Chloride Solution,** N/10.—Dissolve 5.85 grams of pure, dry, recrystallized sodium chloride in distilled water and dilute to 1 liter.

(*c*) **Potassium Chromate Indicator.**—Dissolve 10 grams of pure crystals, free from traces of chlorine, in 100 cc. of distilled water.

To test the accuracy of the standard silver solution fill a burette with the same and then measure into a beaker 25 cc. of the salt solution and add a few drops of the indicator. Now slowly run solution from the burette into the beaker, shaking the latter meanwhile, and continue until the curdy white precipitate shows a tinge of red from the presence of a little chromate formed. Exactly 25 cc. of the silver solution should be needed for this. One cc. of the silver solution so made precipitates 3.55 milligrams of chlorine or shows 5.85 milligrams of sodium chloride.

This method cannot be applied directly to the urine because of its yellow color which obscures the end reaction, and because, also, of the presence of certain organic matters which interfere to some extent. Therefore proceed as follows: Measure out accurately into a platinum or porcelain dish 10 cc. of the urine and add about 2 grams of potassium nitrate and 1 gram of dry sodium carbonate, both free from chlorides. Evaporate to dryness on the water-bath and then heat over

the free flame, at first gently and finally to a higher temperature until the mass fuses. The organic matter is destroyed by the nitrate, leaving finally a white molten residue. Allow it to cool, dissolve in water and add enough pure nitric acid to give a faint acid reaction. This destroys the carbonate. The slight excess of nitric acid must in turn be neutralized and this is done by adding a little precipitated and thoroughly washed (chlorine-free) calcium carbonate. Pour the solution now into a flask, rinse the dish thoroughly, and add the rinsings to the liquid in the flask. Next add 2 drops of the chromate indicator and then the $\frac{N}{10}$ silver solution, until the faint red of silver chromate mixed with the chloride appears, showing the end of the titration. If the urine contains sugar or albumin in more than traces evaporate and heat with the sodium carbonate first and then add the nitrate, a little at a time, to the charred mass to avoid too explosive an oxidation. The titration of the residue from the urine is therefore similar to the process by which the correctness of the silver solution was determined above. If in the titration 22 cc. of the silver solution were used we have $22 \times 3.55 = 78.10$ milligrams of chlorine in the 10 cc. of urine, corresponding to 128.7 milligrams of sodium chloride. The sodium carbonate should not be omitted in this method as without it there is danger of loss of chlorine by volatilization. With care it gives excellent results but at present is not as generally employed as is the next one.

**Volhard's Method.**—We have here a method by which the chlorine in urine can be quickly and accurately

determined without fusion. The principle involved in the process is this. If to a chloride solution a definite volume of standard silver solution be added, and this in excess of that necessary to precipitate the chloride, the amount of this excess can be found by another reaction, subtracted and leave as the difference the volume actually needed for the chloride. The reaction for the excess depends on these facts. A thiocyanate solution gives with silver nitrate solution a white precipitate of silver thiocyanate, AgSCN. It also gives with a ferric solution a deep red color due to the formation of soluble ferric thiocyanate, $FeS_3(CN)_3$. If the silver and ferric solutions are mixed and the thiocyanate added the second reaction does not begin until the first is completed; that is, the silver must be first thrown down as white thiocyanate before a permanent red shade of ferric thiocyanate appears. The presence of silver chloride interferes but slightly with these reactions. Therefore, if we have a thiocyanate solution of definite strength we can use it with the ferric indicator to measure the *excess* of silver used after precipitating the chlorine of a solution.

The reaction between silver nitrate and a thiocyanate is expressed by the following equation:

$$AgNO_3 + NH_4SCN = AgSCN + NH_4NO_3.$$

For 16.99 milligrams of the silver nitrate we use 7.6 milligrams of the thiocyanate. In this method the standard solutions required are

(*a*) **Standard Silver Nitrate Solution,** N/10. — Made as before with 16.99 grams of the fused salt to the

liter. As the solutions are used with nitric acid present, the standard can also be made by weighing out accurately 10.79 grams of *pure* silver and dissolving it, in a flask, in pure nitric acid. Most of the excess of nitric acid is removed by evaporation, and air is blown through to drive out nitrous fumes. The solution is cooled and diluted to 1 liter.

(*b*) **Standard Thiocyanate Solution,** N/10.—This may be made of the potassium or ammonium salt, but the latter is more commonly used. Weigh out about 7.7 grams of the pure crystals, dissolve in water and make up to 1 liter. Determine the exact strength as explained below. The true weight cannot be weighed out directly because the otherwise pure salt is frequently a little moist, and because further the salt, pure to begin with, undergoes frequently a slight change on standing.

(*c*) **Ferric Solution as Indicator.**—Use for this a nearly saturated solution of ammonium ferric sulphate (ferric alum) free from chlorine.

To find the exact strength of the thiocyanate solution proceed as follows: Measure into a flask or beaker 25 cc. of the $\frac{N}{10}$ silver solution and add to it 2 or 3 cc. of the ferric indicator. This gives some color and a slight opalescence. Now add about 2 cc. of pure strong nitric acid, which removes the color and clears the mixture. Into this, from a burette, let the thiocyanate solution flow, a little at a time, shaking after each addition. A red color appears temporarily, but vanishes on shaking. After a time this red disappears more slowly, which shows that the end of the reaction

is near. The burette solution is therefore added more carefully, best by drops, until at last a single drop is sufficient to give a permanent reddish tinge. Something less than 25 cc. should be used for this. Repeat the test and if the same result is found dilute the thiocyanate solution so as to make 25 cc. of the volume used in the titration. For instance, if 24.2 cc. were required 900 cc. of the solution may be diluted in this proportion:

$$24.2 : 25 :: 900 : x \quad \therefore \quad x = 929.8.$$

We have now a standard thiocyanate solution corresponding exactly to the silver solution. To test it further and illustrate its use with chlorides measure out 25 cc. of the $\frac{N}{10}$ sodium chloride solution described a few pages back, and add to it, from a burette, exactly 30 cc. of the silver nitrate solution, then the ferric indicator and the nitric acid as given above. Shake the mixture and filter it through a small filter into a clean flask or beaker. Wash out the vessel in which the precipitate was made with about 20 cc. of pure water, pouring the washings through the filter. Then wash the filter with about 20 cc. more of water allowing the washings to mix with the first filtrate. This mixed filtrate contains all the silver used in excess of the chloride. Now bring it under the thiocyanate burette and add this solution until a reddish tinge becomes permanent. Exactly 5 cc. should be necessary for this.

The chlorides of the urine may be treated in about the same manner. To a measured volume of the urine, usually 10 cc., an excess of silver nitrate solution is

added, 25 cc. with most urines is enough, and then the indicator and acid. The mixture is filtered, the precipitate washed, and in the filtrate the excess of silver is found by thiocyanate as above. But it occasionally happens that the addition of nitric acid to the urine develops a red color which obscures the end reaction. To avoid this the urine titration should always be made in the following manner:

To 10 cc. of urine add 2 to 3 cc. of pure strong nitric acid, then the ferric indicator and three drops of a saturated, chlorine-free, solution of potassium permanganate. This gives at first a very deep red color, but it soon fades and with it the urine colors, by oxidation. It is not well to add more of the permanganate than here given.

To the yellow solution add now 25 cc. of the standard silver solution, shake well, and filter. Wash the beaker and filter thoroughly, as above described, and in the mixed filtrate and washings find the excess of silver. If in doing this the first drops of the thiocyanate solution added produce a deep red color it shows that too little silver had been used in the first place. Make, therefore, a new test, using more silver nitrate solution, 30 to 50 cc.

To illustrate, if we use for 10 cc. of urine, 25 cc. of silver nitrate, then 3.4 cc. of the thiocyanate, $25 - 3.4 = 21.6$, the amount of silver nitrate solution actually needed for the chlorides. $21.6 \times 3.55 = 76.68$ milligrams of chlorine in the 10 cc. of urine, which corresponds to 126.36 milligrams of NaCl in the 10 cc. or to 12.636 grams per liter. If the urine tested had

a specific gravity of 1.020, this would equal 1.24 per cent.

### Sulphates

Sulphur occurs in urine in several classes of compounds. It is most abundant in the ordinary sulphates, as sodium sulphate, potassium sulphate, and others, and is found also in the so-called ethereal sulphates or alcohol sulphates, of which the combination with phenol is a good illustration. These are all normal products, but the ethereal sulphates may be greatly increased pathologically.

The total amount of sulphuric acid excreted daily, expressed as $SO_3$, amounts to about 2 grams; approximately one-tenth may be considered as held in ethereal combinations, and the rest in the mineral sulphates. Many foods contain traces of sulphates and these traces are excreted as such; but the larger part of the excreted sulphuric acid must be considered as formed from the sulphur in the proteids consumed as diet. Analyses show that all true proteids contain between 1 and 2 per cent. of sulphur; this in the body becomes oxidized to the sulphate form and is eliminated by the urine. Experiments on many individuals have shown that with a meat diet, in which proteids are, of course, relatively abundant, the amount of sulphuric acid excreted is increased, while with a diet of fruits and vegetables, with relatively low proteids, the acid is diminished.

The amount of ethereal sulphuric acid found in the urine is a rough measure of the extent of putrefactive changes taking place in the body. Some of these pu-

trefactive changes are normal in the intestines and are in constant operation; the formation of urinary indican may be explained in this manner. Indol is produced by putrefaction, and this oxidizes to indoxyl, $C_8H_6NOH$. The latter, in turn, combines with sulphuric acid to yield the ethereal sulphate, indican, $C_8H_6NHSO_4$ or $C_8H_6NKSO_4$. Under pathological conditions, as in certain fevers, in peritonitis, in carcinoma of the stomach or intestines, the extent of putrefaction is greatly augmented, consequently there is an increased production of indol and the allied body, skatol. Possibly the larger part of these bodies is eliminated by the feces, but another portion is absorbed by the blood and oxidized. This oxidized part combines with sulphuric acid or acid sulphates in the blood to be subsequently excreted in the forms mentioned. Under such circumstances we find in the urine an increased amount of organic sulphates with a diminished amount of the mineral sulphates. Practically the same increase in ethereal sulphates is observed after the use of certain medicaments containing phenols or similar bodies. In cases of poisoning by common phenol there may be a nearly complete disappearance of the mineral sulphates from the urine. The consumption of certain foods and condiments, rich in aromatics, is followed by a like change in the proportion of excreted ethereal sulphate.

It will be recognized, therefore, that at times the determination of sulphates may have considerable clinical importance. Unfortunately, we have no method by which these determinations may be quickly

made and with accuracy; but the qualitative determination of an increase in the organic sulphates may be readily made after a little practice. The reaction depends on these principles: Barium chloride produces an immediate precipitate in a solution containing a sulphate, with or without the addition of acetic acid. In a solution of an ethereal sulphate made acid with acetic acid, barium chloride does not produce a precipitate, even after moderate heating, but if hydrochloric acid be now added and the mixture be warmed a precipitate will form and settle out. Therefore, if we have together two sulphates, for instance, potassium sulphate and phenyl-potassium sulphate, $K_2SO_4$ and $C_6H_5KSO_4$, they may be separated by precipitating the sulphate of the $K_2SO_4$ first, then that of the other after decomposing it by hot hydrochloric acid, which produces phenol and acid sulphate:

$$C_6H_5KSO_4 + H_2O = C_6H_5OH + KHSO_4.$$

### Detection of Sulphates in Urine

To show the presence of the sulphates in urine we may proceed in this way: To 50 or 100 cc. of urine, filtered, if necessary, add enough acetic acid to impart a good acid reaction. This will prevent the precipitation of phosphates and carbonates. Then add barium chloride in small amount and allow the mixture to stand for the separation of the precipitate of barium sulphate. Filter this off after a time and to the filtrate add a few cubic centimeters of pure dilute hydrochloric acid and boil. If a new precipitate forms this points to the presence of the organic sulphates.

If the urine contains oxalate a precipitate of barium oxalate may form with the first barium sulphate, but as the oxalate is soluble in hydrochloric acid the first precipitate may be treated with this acid to see whether it is soluble or not. If the urine contains albumin this must be separated by coagulating with a little acetic acid, warming, and filtering. The tests are then made on the filtrate. As the normal amount of organic sulphates in the 50 or 100 cc. of urine taken for a qualitative test is small, the precipitate will be far from heavy, but if the physician, by examination of a sufficient number of samples, once makes himself familiar with this normal appearance he will soon learn to detect an abnormally large amount without resorting to more complex methods. This skill can be acquired only by experiments on many samples of healthy urine.

### Determination of Sulphates

The volumetric processes for the measurement of sulphates in urine are not convenient and, besides, are not extremely accurate. The usual gravimetric process, while accurate, is one which can be carried out only by a chemist in a properly equipped laboratory. It is therefore not clinically available and cannot be described at length in this place. It may be simply stated that the precipitates formed from a given volume of urine, as in the qualitative test, are carefully collected on filters, thoroughly washed, dried, and weighed. From this weight of $BaSO_4$ the acid may be calculated as $SO_3$ or in any other form as desired. For the details of methods of gravimetric analysis special works on analytical chemistry must be consulted.

# CHAPTER VIII

## AMMONIA, XANTHIN AND ALLIED BODIES, AND CREATININ

### Ammonia

A small amount of ammonia is present in combination in fresh normal urine, while in old urine the amount may be large from the decomposition of urea. With this we have nothing to do. The normal occurrence of ammonia in urine may be credited in part to traces of ammoniacal salts taken with the food, but principally to the product formed by proteid disintegration. If the theory of the formation of urea from ammonium carbonate be correct, then the small amount of ammonia found in the urine, in excess of that taken directly with the food, could be referred to that left over by a failure to complete this reaction in the body:

$$(NH_4)_2CO_3 = CO(NH_2)_2 + 2H_2O.$$

It has been shown that ammonia in the urine is increased by a flesh diet, as is also urea, and that by a vegetable diet it is low. The average normal amount is about 0.7 to 0.8 gram daily, but the amount may be increased to 1.5 grams daily, or sink to 0.3 or 0.4 gram.

Pathologically there seems to be a marked increase of excreted ammonia in fevers and the amount is usually found to vary with the greatly increased acid excretion. This has been noticed in diabetes mellitus

where a marked elimination of oxybutyric acid or diacetic acid is sometimes observed. The determination of excreted ammonia may, therefore, have practical value in diagnosis as bearing on the progress of the pathological condition.

### Determination of Ammonia

As ammonia is always present in urine in some amount, qualitative tests have little value, and we proceed immediately to quantitative methods. As the ammonia present is in combination as a salt it must be liberated by the action of a strong alkali, but in the choice of one for this purpose we are limited by the fact that the hydroxides of sodium and potassium have a decomposing action on urea and other nitrogenous bodies in urine and cannot, therefore, be used. Milk of lime is free from this objection and may be employed. The experiment is so arranged that the liberated ammonia may be absorbed by a measured volume of standard sulphuric acid, the amount of this neutralized being the measure of the ammonia absorbed:

$$H_2SO_4 + 2NH_3 = (NH_4)_2SO_4.$$

98 parts by weight of the acid correspond to 34 parts of ammonia, $NH_3$.

The test is best made in this manner: Measure 25 to 50 cc. of the fresh urine into a shallow glass dish with a flat bottom resting on a ground glass plate. A so-called crystallizing dish may be used with advantage. On this support a triangle of glass, and on this triangle place a second glass dish, preferably smaller

than the first. Measure into this top dish 10 cc. of normal sulphuric acid and then add to the urine 10 to 20 cc. of good fresh milk of lime. Cover the whole with a bell-jar, as shown in the illustration, and allow to stand several days. In this time the milk of lime expels the ammonia from the urine. The rim of the bell-jar must be ground to fit the plate perfectly and in addition to this, should be rubbed with a little tallow.

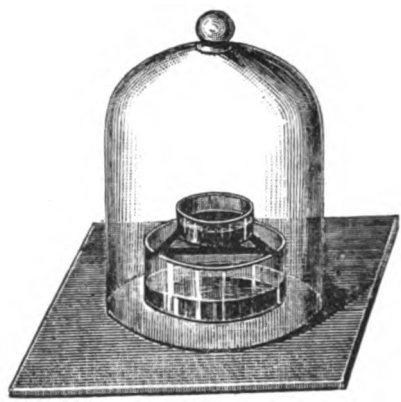

Fig. 7.

As the ammonia cannot escape into the air it is absorbed by the normal sulphuric acid, and after a time may be measured by titration. It is necessary to allow the apparatus to stand three or four days to insure complete absorption, as part of the liberated ammonia collects at first with a deposit of moisture on the inner walls of the bell-jar, and is given up from here rather slowly. When absorption is supposed to be complete the bell-jar is removed and the acid in the upper dish

titrated with weak standard alkali, preferably fifth normal sodium hydroxide, after adding methyl orange as indicator. The preparation of the standard sulphuric acid and the standard alkali is given in the appendix. Before titrating, it is well to rinse the sides of the bell-jar down with a little pure water and add the rinsings to the acid.

In illustration of the calculations to be made in this test assume the following case: Take 50 cc. of urine and 10 cc. of the normal sulphuric acid. Each cubic centimeter of the acid contains 49 milligrams of actual $H_2SO_4$ and will absorb and combine with 17 milligrams of $NH_3$. Suppose now that at the end of the experiment, after adding methyl orange to the acid in the glass dish, we add from a burette 32.5 cc. of the fifth-normal sodium hydroxide solution to reach the point of neutrality (that is, to combine with the acid in excess of that neutralized by the ammonia), this amount of alkali is equivalent to 6.5 cc. of normal alkali and neutralizes, therefore, 6.5 cc. of the normal acid. 3.5 cc. of the acid must have been neutralized by ammonia. Now, as each cubic centimeter of acid will absorb and neutralize 17 milligrams of $NH_3$, it follows that the total amount of ammonia present in the 50 cc. of urine must have been

$$3.5 \times 17 = 59.5 \text{ milligrams,}$$

or 1.19 grams to the liter.

This test must always be made on fresh urine, as that which has stood contains an excess of ammonia from the bacterial fermentation of urea. When it is desired to save the day's urine for examination it is

necessary to add a little phenol to the collecting vessel, enough to make about 2 per cent. of this body present in the volume collected.

### The Xanthin Bases

Normal urine contains a number of complex nitrogenous bodies which in composition, and to some extent in chemical behavior, are closely allied to uric acid. Of these substances the following are known:

| | |
|---|---|
| Xanthin | $C_5H_4N_4O_2$ |
| Heteroxanthin | $C_6H_6N_4O_2$ |
| Or methylxanthin | $C_5H_3N_4O_2CH_3$ |
| Paraxanthin | $C_7H_8N_4O_2$ |
| Or dimethylxanthin | $C_5H_2N_4O_2(CH_3)_2$ |
| Guanin | $C_5H_5N_5O$. |
| Hypoxanthin (sarkin) | $C_5H_4N_4O$. |
| Adenin | $C_5H_5N_5$ |
| Carnin | $C_7H_8N_4O_3$ |

Uric acid is represented by the formula, $C_5H_4N_4O_3$.

These bodies are all distinguished by their very slight solubility in water, by their ready solubility in mineral acids, with which they form crystalline combinations of slight stability, and by precipitates which they give with solutions of many heavy metallic salts. Some of these precipitates are formed in extremely dilute solution. Under the tests for uric acid it was explained that in the precipitation of this acid by ammoniacal silver nitrate, Haycraft's method, the results are slightly inaccurate because of the coprecipitation of other bodies. The xanthin bases are the products having the greatest effect here, as they all combine

with the silver or silver oxide of the alkaline solution.

The amount of these bodies present in normal urine is small; xanthin is the most abundant and of this the daily excretion is about 30 milligrams. For the detection of some of the bases it is necessary to operate on large quantities of urine.

For the clinical separation of the xanthin bodies no convenient method is available. By operating on a large volume of urine they may be obtained in a silver precipitate along with uric acid. This precipitate is thoroughly washed, mixed with water, and finally decomposed by hydrogen sulphide to separate the silver; after filtering off the sulphide and concentrating the filtrate to a small volume, the uric acid separates from the other substances almost completely. On filtering again, a solution is obtained from which the bases may be thrown down by several special methods. Instead of using ammoniacal silver solution as a precipitant phosphotungstic acid along with hydrochloric acid may be employed. This gives a bulky precipitate containing the bases and uric acid. This precipitate is washed with weak sulphuric acid until free from chlorine, drained and heated with a solution of barium hydroxide which forms an insoluble urate and brings the bases into solution. After filtering, the excess of baryta may be separated from the filtrate by a little sulphuric acid. On again filtering a solution is obtained from which the bases may be thrown down by ammoniacal silver nitrate. For the identification of the individual bases in this precipitate the reader is referred to Neubauer and Vogel's " Urine Analysis."

### Creatinin

This product, having the formula $C_4H_7N_3O$, occurs normally in urine and is excreted to the amount of 1.5 to 2 grams daily. It is, therefore, more abundant than uric acid. As it is readily soluble in water and acids it escapes detection except when looked for by special reagents. In weak solutions it is precipitated by phosphotungstic acid, phosphomolybdic acid, picric acid, and especially by solutions of several heavy metallic salts. The precipitate given with a neutral solution of zinc chloride is the most characteristic. It gives certain color reactions also. The following test may be applied to urine. If acetone is present it must be expelled by heat. To about 25 cc. of this urine add half a cubic centimeter of a dilute solution of sodium nitroprusside made alkaline with caustic soda. With this the urine gives a ruby-red color, fading to yellow. Then add acetic acid in slight excess and warm. A green color soon appears, deepening finally to blue.

To obtain a characteristic precipitate with zinc chloride some preliminary treatment is necessary, which may be applied in this manner. To about 250 cc. of urine add milk of lime to alkaline reaction, and then a few cubic centimeters of a 10 per cent. solution of calcium chloride. This gives a bulky precipitate of phosphates and urates which is filtered off. The filtrate is made neutral with a small amount of acetic acid and evaporated to the consistence of a sirup on a water-bath. This sirup is treated with strong alcohol (98 per cent.) to dissolve the creatinin and leave salts

in insoluble form. After standing six hours the mixture is filtered and to the filtrate a strong solution of pure chloride of zinc in alcohol is added. The mixture is stirred and allowed to stand two days, in which time a crystalline precipitate of the double salt settles out. This has the composition $(C_4H_7N_3O)_2\ ZnCl_2$. The yield of this precipitate is somewhat increased by adding a small amount of sodium acetate solution before adding the zinc chloride. This insures precipitation from a solution free from stronger acids. The precipitate is to be washed on a filter with alcohol, and a portion may then be examined by the microscope. Rosettes or bundles of fine needles are usually obtained.

# CHAPTER IX

## THE SEDIMENT FROM URINE

Urine is frequently cloudy when passed and on standing deposits a sediment of the substances imparting the cloudiness. Other urines which may appear perfectly clear at first also throw down deposits after a time. This is always the case with urine allowed to stand long enough to undergo alkaline fermentation, when a precipitate of phosphates forms. The deposit is frequently caused by a change of temperature. Warm voided urine holding an excess of urates may be perfectly clear, but becomes cloudy as its temperature goes down with the formation of a light reddish sediment. This is a perfectly normal action, and indeed most sediments may be considered in the same light. Urine containing a deposit is not necessarily pathological.

There are conditions, however, in which the sediment is an indication of abnormality, and its examination becomes important clinically. Certain sediments are pathological because of their origin, others because of their amount. For instance, blood and pus corpuscles, casts of the uriniferous tubules of the kidney and a few other forms are not found normally in urine, and their presence is of importance, whether observed in large or small quantity. Sediments containing phosphates, uric acid and

urates, calcium oxalate and other salts, are common enough and usually attract no attention, but if the amount of these deposits is very large there may be attached to them clinical significance and they deserve study.

In the examination of a sediment it is necessary to allow the urine to stand long enough to deposit the important forms it may contain, which may require twenty-four hours or more. For the deposition of a sediment the urine should be left in a place with an even temperature, preferably not above 15° C. A low temperature favors the precipitation of urates, while decomposition may begin if the temperature be allowed to go up. Some of the light organic forms have a specific gravity so little above that of the urine that they may remain a long time in suspension. It is important, therefore, to allow plenty of time for these to settle. If the weather is warm and there is no good means at hand for keeping the temperature of the urine down until the examination can be made, or if for any reason this must be delayed for some days, it is well to add some preservative to the urine; *i. e.*, something to prevent fermentation. Many substances have been suggested for this purpose, some of which are very objectionable inasmuch as they form precipitates which often obscure what is sought for. Chloroform is the simplest and at the same time one of the best substances which can be added.

To 100 cc. of the urine to be set aside for tests add three or four drops of chloroform and dissolve by shaking. It is not well to add more than this as there is

danger of leaving minute droplets undissolved, and these are confusing in the subsequent examination. The chloroform may be applied in the form of aqueous solution. Add about 10 grams of chloroform to a liter of distilled water and shake thoroughly; about three-fourths will dissolve at the ordinary temperature; 25 cc. of this saturated solution may be added to 100 cc. of the urine to be examined, which is then allowed to stand as before.

Recently, formaldehyde has come into use as a urine preservative and is applied as is the chloroform. It must be remembered that both of these substances are reducing agents, and therefore should not be used with urine to be tested for sugar.

After the deposit has settled pour off the supernatant liquid very carefully and by means of a small pipette with a *coarse* opening transfer one or two drops to a *perfectly clean* glass slide. Clean a cover glass with *great care* and by means of small brass forceps lower it on the drop of liquid in such a manner as to exclude air bubbles. This can be done by lowering it inclined to the slide, not parallel with it, so as to touch the liquid on one side first. In settling down, the cover now pushes the air in front of it and gives a field generally free from bubbles. The slide is then examined under a microscope with a magnifying power of 250 to 300 diameters. Either natural or artificial light may be used, but it must not be very bright. A very common mistake in the examination of urinary sediments by the microscope is to employ so high a degree of illumination that the lighter and

nearly transparent bodies are completely overlooked.

Recently, centrifugal machines have been introduced which may be employed to settle the urine. The latter for this purpose is placed in strong test-tubes which are caused to rotate so rapidly that all suspended matters are thrown to the bottom of the tubes, these being hung by the neck in such a manner that in ro-

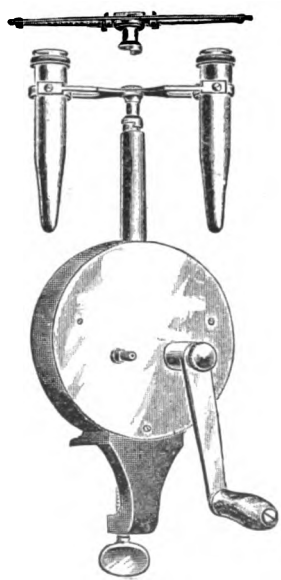

Fig. 8.

Fig. 8 bis.

tation the bottom flies up and outward. Some of these rotating machines are operated by hand, others by water power or electricity. A very convenient form run by a small water motor is now to be had from dealers. It is simply necessary to attach the motor by means of a rubber tube to a faucet delivering water

under ordinary city pressure to obtain all the power necessary.

Where the current furnished for electric lighting by the incandescent system is available, centrifugal machines operated by small electric motors are even more convenient. The cut to the right shows a small machine which has given excellent results. The wires from the motor in the base of the machine are attached by a socket in place of the common incandescent lamps now everywhere used. The cut to the left shows a hand-power machine.

A very high velocity is attained in these machines, by aid of which the deposit may be secured in minutes instead of hours. The sediment is left in a very concentrated condition in the bottom of the tube, and from it the supernatant urine can be poured much better than when it precipitates in a beaker or bottle in the usual manner.

Sediments from urine are commonly classed as *organized* and *unorganized*, these divisions being then subdivided according to various plans. The important forms under each division are shown in the following schemes:

**Organized Sediments.**

- Blood corpuscles.
- Mucus and pus corpuscles.
- Epithelium from various locations.
- Mucin bands, or threads.
- Casts of the uriniferous tubules.
- Spermatozoa.
- Fragments of cancer tissue.
- Fungi.
- Certain other parasites.

**Unorganized Sediments.**

- Uric acid.
- Various urates.
- Leucin and tyrosin.
- Cystin.
- Cholesterin.
- Fat globules.
- Hippuric acid.
- Calcium carbonate.
- Calcium phosphate.
- Calcium oxalate.
- Magnesium phosphates.

In addition to these there are often found in the urine certain bodies whose presence must be called accidental; for instance, hairs, fibers of cotton, silk or wool, starch granules, bits of wood, mineral dust, etc. Some of these will be referred to later.

## Organized Sediments

### Blood Corpuscles

Urine containing blood presents a characteristic appearance easily recognized, unless it be present in very small quantity. If the reaction of the urine is acid the color is generally dark; but if alkaline the shade is inclined to reddish. Blood corpuscles enter the urine from several different sources and their presence is usually a pathological indication, but not always, as they may come, for instance, from menstruation. The kidneys, or their pelves, the ureters, the bladder, the urethra, the vagina, or the uterus may be the seat of the lesion from which the blood starts, and its appearance sometimes gives a clue to its origin.

Fresh blood corpuscles are clear in outline and show distinctly their biconcavity. But corpuscles which

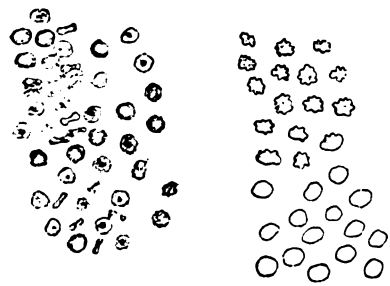

Fig. 9. Human blood corpuscles, 400 diameters.

have been long in contact with the urine become much swollen, less distinct in outline, often biconvex, or nearly spherical even, and lighter in color. As long as the reaction of the urine is acid the corpuscles remain comparatively fresh in appearance but with the beginning of the alkaline reaction disintegration and loss of color soon set in.

The microscopic recognition of blood in urine is easy enough if it is not too old. The fresh, red corpuscles of human blood have a mean diameter of about 0.0077 mm., but when swollen by absorption of water they are somewhat larger. When seen on edge they appear as shown at the left in the figure above. If presenting the flat side to the eye, they appear as disks whose centers grow alternately light and dark by changing the focus of the instrument. In old urine, especially with alkaline reaction, they appear as granulated spheres, shown at the left of the figure. In all cases the color is more or less yellowish. It is gen-

erally assumed that the paler washed-out corpuscles come from lesions higher up, from the pelvis, or kidney even, while the brighter fresh blood suggests a lesion nearer the point of discharge; that is, from the bladder or urethra. This is pretty certain to be the case if the blood is discharged but little mixed with the urine and settles rapidly as a distinct mass.

### Mucus and Pus Corpuscles

These are white corpuscles somewhat larger than the red blood corpuscles and spherical in outline. The term *leucocyte* is frequently applied to these as well as to the so-called white corpuscles of blood. Their size varies greatly but the average diameter may be given as 0.009 mm. All these corpuscles present, when fresh, a slightly granular appearance and occasionally show one or more nuclei. The addition of a little acetic acid to the sediment brings the nucleus out distinctly so that it may be seen under the microscope as a characteristic appearance.

Mucus corpuscles in small number are normally present in urine, but pus corpuscles enter the urine as a constituent of pus itself which is an albuminous product discharged from suppurating surfaces and not normal. In a former chapter it was shown that the reactions of mucus and albumin are distinct, but urine containing pus always affords reactions for albumin. Pus in urine tends to form a sediment at the bottom of the containing vessel, and may be recognized by the following method:

**Donne's Test.**—Pour the urine from the sediment

and add to the latter an equal volume of thick potassium hydroxide solution, or a small piece of the solid potassa. Stir with a glass rod. The strong alkali converts the pus into a thick viscid mass closely resembling white of egg. Sometimes this is so thick that the test-tube containing it can be inverted without spilling it. In alkaline urine this glairy mass is sometimes spontaneously formed.

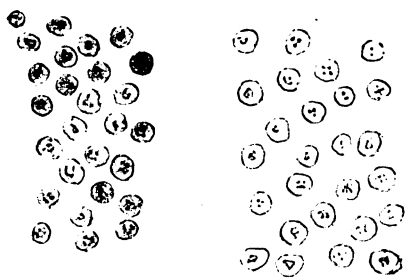

Fig. 10. Pus corpuscles, 400 diameters.

The appearance of the mucus or pus corpuscles in urine depends largely on the concentration of the latter. In urine of low specific gravity the corpuscles absorb water and swell to larger size than normal, while in a highly concentrated urine they may give out water and become reduced in size and shrunken in appearance.

To recognize them under the microscope transfer a few drops of the sediment to a slide, and cover as usual. If the nuclei are not distinct place a drop of diluted acetic acid on the slide at the edge of the cover glass. Part of the acid will flow under the cover and mix with the urine. As it does this the clearing up of the

corpuscles, with appearance of the nuclei, can be very easily followed. Urine containing much pus is white and milky. The same appearance is often noticed with an excess of earthy phosphates, but the latter clear up with acids while the pus does not.

### Epithelium Cells

Epithelium cells from different sources may appear normally in the urine, and the light cloud which separates from normal urine on standing consists chiefly of these cells. When present in small amount this epithelium has usually no clinical importance, as it easily finds its way into the urine from the bladder, vagina, or urethra. An abundance of cells from these

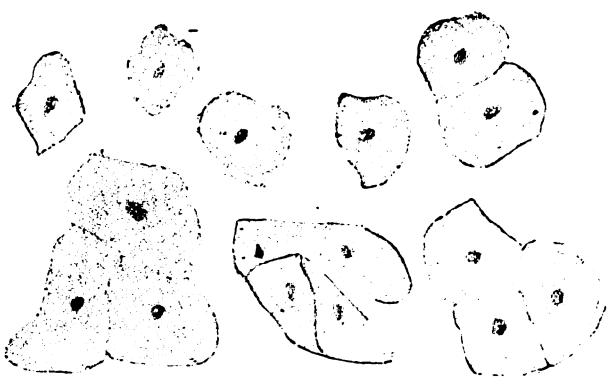

Fig. 11. Scaly and spherical epithelium.

organs would, however, be considered pathological, pointing to a catarrhal condition.

Unfortunately, it is not possible in all cases to determine the source of the cells, as found in urine,

partly because cells from different localities have frequently the same general appearance, and partly because, owing to immersion in the urine, they become greatly changed from what they are in the tissue as shown by the microscopic study of sections. It is customary to make three rough divisions of the cells as found in the urine:

1. Spherical cells. 2. Columnar or conical cells. 3. Flat or scaly cells.

The spherical cells are probably normally much

Fig. 12. Conical and spherical epithelium.

flattened, but by absorption of water they become swollen and globular. These cells may be derived from several sources, as from the uriniferous tubules or from the deeper layers of the lining membrane of the pelvis of the kidney, or the bladder, or the male urethra.

These cells have a well-defined nucleus resembling that of a pus cell. But they are much larger, and

besides show the nucleus without addition of acid. In nephritis, or other structural diseases of the kidney, these round cells are found along with albumin, and their recognition is then a matter of importance as indicating a breaking down of the tubular walls. Sometimes these cells form a variety of tube cast, to be described later. But it must be remembered that we cannot distinguish with certainty between the cells from the tubules and those from the other localities mentioned.

Conical cells come generally from the pelvis of the kidney, from the ureters and urethra. Some of these cells are furnished with one or two processes, and are broad in the middle and taper toward each end, while the others are broad at the base and taper to a point.

The large flat cells come from the vagina or bladder, and it is generally impossible to distinguish between them. Sometimes they are very nearly circular, sometimes irregularly polygonal in outline. Sometimes the vaginal epithelium is found in layers of scales, which appear thicker and tougher than the cells from the bladder, which occur singly.

What was said about the decomposition of blood or pus cells in urine obtains also for the various epithelium cells. In acid urine they may maintain their distinct outlines many days, but in alkaline secretion they soon undergo disintegration, which makes their recognition practically impossible. In general the greatest importance attaches to the cells from the tubules of the kidney. The presence of albumin in more than minute traces in the urine would suggest

that any smaller spherical cells present may have had their origin in the kidney rather than in the bladder or male urethra. In general it may be said that urine containing large numbers of the smaller, round tubule cells with albumin will also show casts.

### Mucin Bands

Urine containing much mucus sometimes exhibits a deposit consisting of long threads or bands, curved and bent in every direction. These bands are important because they are sometimes confounded with the

Fig. 13. Mucin bands or threads.

tube casts to be described next. They can be produced in urine highly charged with mucus by the addition of acids, and appear therefore sometimes spontaneously when the urine becomes acid. These threads are sometimes covered with a fine deposit of granular urates and then bear some resemblance to granular casts. In general, however, they are relatively longer and narrower than the true casts of the uriniferous

tubules. The mucin threads can occur, and frequently do occur, in urine entirely free from albumin, while true tube casts are usually associated with albumin, although not always, as will be explained below. The length and shape of the mucin threads may generally be relied upon to distinguish them from true casts.

## Casts

The structures properly termed casts are seldom found in urine which does not contain albumin. They are formed in the uriniferous tubules, and, to a certain extent, are "casts" of portions of the same. Their specific gravity differs but little from that of the urine, for which reason they remain long in suspension. It is therefore necessary to allow the urine to stand some hours at rest, over night or longer, before attempting an examination, if a centrifuge is not at hand.

Casts of the uriniferous tubules rarely appear in normal urine and their recognition is therefore a matter of the highest importance in diagnosis. Much has been written on the subject of the origin of these bodies in the kidney and several theories have been advanced to account for their formation and chemical constitution. Most of this discussion would be out of place in a work like the present dealing mainly with questions of analysis, but enough will be given to aid the student in his examinations. It must be said that few subjects are more perplexing to the beginner than that of their certain recognition, because of the fact that some varieties are so transparent as to be almost invisible, while others are closely resembled by forma-

tions of entirely different nature, not pathological. With practice, however, these difficulties can be surmounted.

Most of the bodies termed casts are formed of organized structures or the remains of such, but another and rather common form consists of crystalline matter, usually uric acid or fine granular urates.

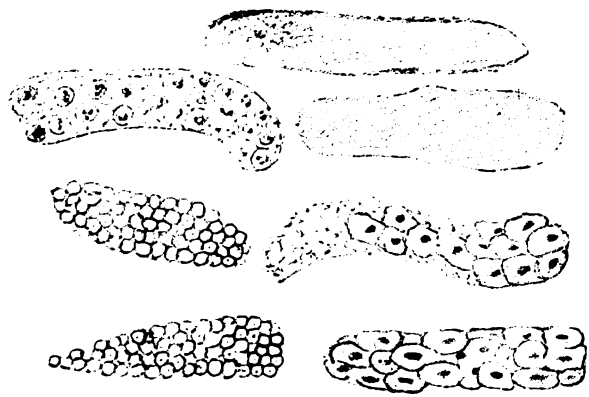

Fig. 14. Epithelium and blood casts; above, a bunch of urates or false casts.

These bunches of urates have no pathological significance and are of frequent occurrence. Urine containing them clears up by heat, and the deposits themselves are dissipated by weak alkali. While it is true that they resemble, to some degree, the so-called granular casts referred to below, there are certain well-defined points of difference. The bunches of urates lack the coherence which can be observed in the true casts, and besides, the granulation is finer and more clearly defined.

The fact that mucin bands occasionally appear covered with a precipitate of granular urates has been referred to. These aggregations are more compact than the loose bunches of urates just mentioned and much

Fig. 15. Blood casts and granular casts.

longer generally. They are also darker and therefore more easily seen than are the casts proper or the urates.

The true casts are made up of matter in which evidence of cell structure or transformation is visible. An accurate classification of these bodies cannot yet be made, and, as said, authors differ regarding the importance of several forms and their origin. But for our purpose it will be sufficient to make the following rough division, which accords in the main with what is found in the text-books of urine analysis:

1. Blood casts.
2. Epithelium casts.
3. Granular casts.
4. Fatty casts.
5. Waxy casts.
6. Hyaline casts.

What are termed blood casts consist of or contain coagulated blood, recognized by the corpuscles. Plugs of this coagulated matter are forced out from the tubules by pressure from behind, and form one of the most characteristic varieties of casts. They are generally very dark in color, and easily distinguished from other matter. A representation of blood casts is given in the preceding cut.

In epithelium casts the characteristic substance is the lining epithelium of the tubule. Sometimes this lining epithelium becomes detached in the form of a hollow cylinder, the walls consisting of the united cells. Again, the coagulated contents of the tubule in passing out may carry the epithelium with it as a coating. In either case a grave disorder of the kidneys is indicated, as acute nephritis, or other disease in which a profound alteration of the internal structure of the organ is involved.

What are termed granular casts, proper, appear in a variety of forms, produced probably by the disintegration of blood or epithelium casts.

There is no uniformity in the fineness of the granulation; sometimes a high amplification is necessary to disclose the structure. Occasionally blood corpuscles, epithelium, fat globules and crystals can be detected in them, and when derived from blood cast disintegration they usually have a yellowish red color, which makes their recognition comparatively easy. In outline they are generally regular, with rounded ends, one of which is somewhat pointed. Frequently, however, they appear to be broken, the ends showing irregular fracture.

Fatty casts contain oil drops produced by some variety of fatty degeneration of the tissues of the kidney. These oil drops may form coherent bunches, or they may be held by patches of epithelium. It also happens that epithelium or granular casts may be partially covered by oil drops. The name, fatty cast, is applied to those in which the fat globules predominate. Along with these globules the microscope sometimes shows crystals of free fatty acids, and probably also of soaps containing calcium and magnesium.

Waxy casts consist of the peculiar matter produced

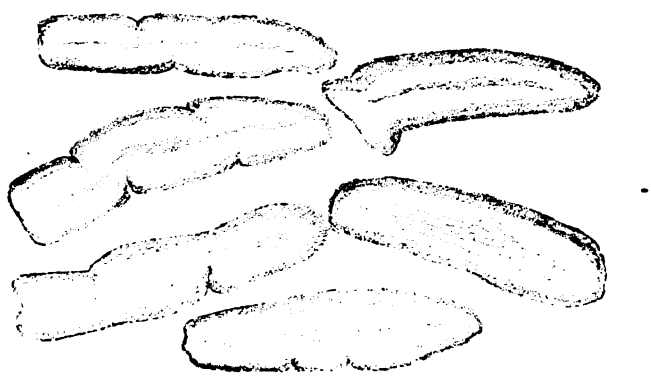

Fig. 16. Waxy and hyaline casts.

by amyloid degeneration of the kidney. They have a glistening wax-like or vitreous appearance, and refract light very strongly. Sometimes they reach a great length, and they frequently are found with blood corpuscles or oil drops on the surface. They have been detected in several renal disorders. Illustrations are given.

True hyaline casts are nearly transparent and hard to see unless the illumination is very carefully managed. To detect them it is often necessary to add a few drops of a dilute solution of iodine in potassium iodide to the sediment. This imparts a slight color which renders them visible.

The hyaline casts seem to be formed by the passage of homogeneous matter from the tubules, leaving the epithelium behind. A cast is rarely perfectly hyaline, as at least an occasional blood corpuscle, fat globule, or epithelium cell will usually be found attached to it. Waxy casts may be looked upon as a special form of hyaline casts. Very imperfect representations are given in the above cut.

In general, it must be said that the representation of these casts on paper is a very difficult matter. Ordinarily they are drawn and printed much too heavy and dark.

Hyaline casts do not necessarily indicate kidney disease, although this is usually the case. They have been found in urine free from albumin and under circumstances not connected with renal disorders.

The preservation of sediments containing casts is unusually difficult because of the nature of the material to be preserved. In urine of the slightest alkalinity their disintegration soon begins, so that the outlines are rendered indistinct, often making identification impossible.

For temporary preservation the addition of chloroform renders as good service as anything else. Many other sediments can be permanently mounted and kept

for future comparison but with casts this can rarely be done.

Beginners are apt to overlook casts in their first examinations. It must be remembered that some of them are nearly transparent and unless brought into proper focus they may not be seen at all. At the outset students usually employ too bright a light in looking for casts. While no specific directions can be given regarding the intensity of illumination best suited to the purpose, this may be said that the light commonly found necessary in studying ordinary histological slides is far too bright to use in the search for casts.

Practice alone, first under the direction of the instructor, will indicate what is proper here.

## Spermatozoa

These minute bodies, as found in the semen of man, have a mean length of about 0.050 millimeter. Nearly one-tenth of this is in the head portion. When observed in recently discharged semen they have a characteristic spontaneous movement by which they are propelled forward rapidly. This motion is soon lost if the semen is diluted with water or similar liquid. Hence, as usually seen in urine, they are entirely motionless. They are found abundantly in the urine of men after coitus or nocturnal emissions, and also in spermatorrhea, when their presence is continuous and characteristic.

In the urine of women they are likewise found after connection, and their detection here has often interest

from the medico-legal standpoint. The proof of rape can often be established by the recognition of spermatozoa.

Although their motion is soon lost in foreign liquids the substance itself of the spermatozoa is not readily destroyed. In this respect they are more permanent, probably, than all other organized structures found in the urine and can be readily distinguished after many

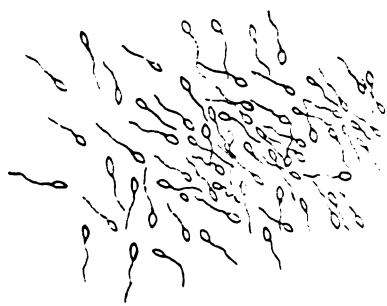

Fig. 17. Human spermatozoa.

days or months even in urine, or in sediments which have become dry.

For their recognition a power of 250 diameters is sufficient, but 350 to 400 diameters is preferable. With this higher amplification it is possible to readily distinguish between spermatozoa and certain fungus growths bearing some resemblance to them.

## Cancer Tissue

Fragments of cancerous and other morbid growths are occasionally met with in the urine, and when certainly recognized are an important aid in diagnosis.

The recognition of cancer cells along with the several kinds of epithelium cells found in urine is difficult and lies somewhat beyond the usual line of urine examinations.

Fig. 18. Cancer cells which have been seen in urine.

Sometimes the identification is comparatively simple because of the unusual shape of the cells, or when they are present in great number, but this is not often the case.

The cut shows some of these cells figured by Beale.

## Fungi

The urine sometimes contains certain fungus growths, the recognition of which is important. These may have entered the urine after voiding, or they may have come from the bladder.

Normal urine when passed is probably free from fungi of all kinds, but in a short time certain organisms enter it from the air or from other sources and become active in producing in it characteristic changes.

The three important groups of fungi, the *schizomycetes* or *bacteria*, the *hyphomycetes* or *molds* and the *blastomycetes* or *yeasts* are represented in the organ-

isms sometimes found in the urine. The conditions under which they are found will be briefly explained.

Of the bacteria the following have been observed:

**Micrococcus Ureæ.**—This is the exceedingly common form found on urine undergoing alkaline fermentation by which urea is converted into ammonium carbonate. It is usually introduced from the air and multiplies very rapidly under ordinary conditions. Nearly all old specimens of urine, unless containing some active preservative, are found infected with this small organism. The micrococci are minute spherical bodies belonging to the suborder *spherobacteria*, and are found separate or in chains. They are the smallest of the organized forms occurring in urine and appear under a power of 250 diameters but little more than points.

While generally finding their way into urine after it has been voided they are occasionally present in the bladder. It is usually held that under such circumstances they have been introduced by a dirty catheter or sound, although cases are on record where this has not been proved.

In the bladder they give rise to alkaline fermentation, so that the voided urine may show ammonium carbonate directly.

**Streptococcus Pyogenes** is a pathogenic form sometimes found in the urine in cases of infectious diseases.

**Sarcinæ.**—The genus *sarcina* is frequently classed with the spherobacteria and several species have been found in urine. The cells are larger than those of

micrococcus ureæ, and are arranged in groups of two or four usually. They are not pathogenic.

**Bacilli.** — Several species of the genus *bacillus* are found in urine in disease. The most important of these are the typhoid bacillus, *bacillus typhi abdominalis*, the tubercle bacillus, *bacillus tuberculosis*, and the bacillus of glanders, *bacillus mallei*. These bacilli occur in urine only during the progress of the corresponding diseases and their detection is of the highest interest. A description of the methods to be followed

Fig. 19. Micrococci and other bacteria.

for the certain demonstration of these bodies is not within the scope of this book, but must be looked for in the laboratory manuals of bacteriology.

**Spirilla.** — Certain species of the genus *spirillum* have been found in urine. The best known of these is the spirillum of relapsing fever, *spirillum Obermeiri*. This is only found rarely and as its habitat is the blood of relapsing fever patients it must enter the urine through a hemorrhage into the kidney. Its form is that of a long, wavy spiral, which makes its detection somewhat easy.

Although not pathogenic it is well to call attention to certain molds which may sometimes be seen in urine. The common blue-green mold, *penicillium glaucum*, is the best known of these, and is occasionally found in urine along with yeast cells. Another mold which has been found in urine is the *oidium lactis*, commonly occurring in milk and butter. It has been observed in fermenting diabetic urine. Both of these fungi enter the urine after voiding. In urine which has stood sometime in a cool place the *penicillium glaucum* sometimes becomes covered with an incrustation of urates or minute crystals of uric acid.

Finally we have yeast cells in urine and sometimes in great numbers. Like other fungi they enter the

Fig. 20. Yeast cells and common mold.

urine from the air and when not very abundant have no significance. In great numbers the yeast cells suggest presence of sugar. The ordinary yeast plant, *saccharomyces cerevisiæ*, is shown, isolated and budding, in the accompanying figure.

## Other Parasites

Besides fungi the urine in rare cases contains other parasites of animal as well as vegetable nature. Some of these enter it accidentally from the air and have no interest, but a few are present in the urine when passed, and are of importance. Among these are certain thread worms, the eggs of worms and hooklets of a species of tapeworm, the *taenia echinococcus.*

These forms can appear in the urine only through rupture from some other organ, and while rare here, are common enough in Egypt and other tropical countries. Urine containing eggs of worms, the worms themselves or fragments, usually contains blood or other evidence of rupture.

# CHAPTER X

## UNORGANIZED SEDIMENTS AND CALCULI

### Unorganized Sediments

#### URIC ACID

Among the more common of the unorganized sediments found in urine this must be mentioned first. As was explained in the last chapter uric acid occurs normally in combination in all human urine.

Some time after its passage urine often undergoes what has been spoken of as the *acid fermentation* by which a precipitate of urates and even free uric acid may appear. This reaction is in no sense due to a ferment process in the ordinary sense of the term, but is probably brought about by a purely chemical double decomposition. Urine contains acid sodium phosphate and neutral sodium urate and it has been suggested that these react on each other according to the following equation:

$$Na_2C_5H_2N_4O_3 + NaH_2PO_4 = NaC_5H_3N_4O_3 + Na_2HPO_4.$$

The precipitate of acid urate settles out and forms a light reddish deposit. If the amount of acid phosphates present is excessive the reaction may go still further, resulting in the precipitation of free uric acid. The well-characterized crystals of uric acid are often found with the sediment of fine urates. Sometimes this liberation and precipitation of the acid takes place

in the bladder, and the urine, as passed, shows the crystals or "gravel." If they are relatively large, which

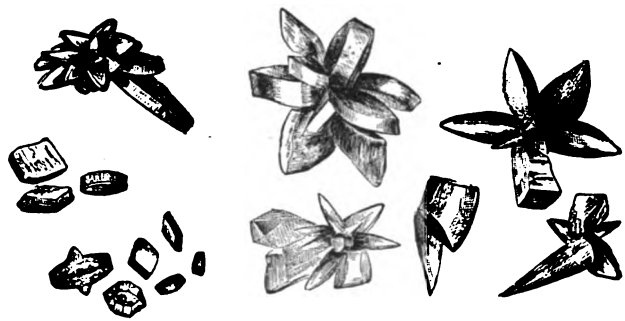

Fig. 21. Uric acid.

is sometimes the case, their passage through the urethra may cause severe pain.

As the illustrations show, uric acid occurs in a great variety of forms. The rosettes and whetstone-shaped

Fig. 22. Uric acid.

crystals are probably the most common, while long spiculated forms are frequently seen. Pure uric acid

is colorless but as deposited from urine it is always reddish yellow, because of its property of carrying down coloring-matters. The crystals are often so large that their general form can be seen by the naked eye; usually, however, they are minute.

Uric acid crystals when once deposited are not readily redissolved by heat, but they go into solution by the addition of alkali. If the urine contains extraneous matter, as specks of dust, bits of hair, cotton or wool fibers, the crystals are very apt to deposit on them.

## URATES

The common fine sediments of urine are usually

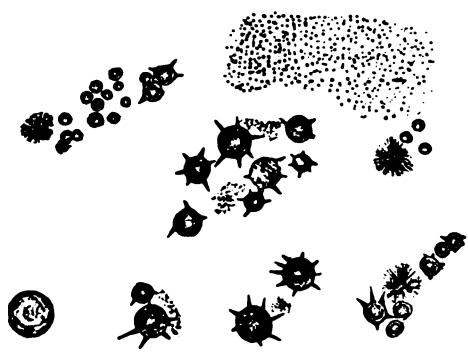

Fig. 23. Common crystalline and granular urates.

urates or amorphous phosphates. They can be most readily distinguished by their behavior with acids and on application of heat. Urates disappear on warming the urine containing them, while a phosphate sediment is rendered more abundant. A urate sediment is little changed by acids, while the phosphates dis-

solve completely if the urine is made acid in reaction with hydrochloric or nitric acid. The acid urates of sodium and ammonium are the most abundant and are shown in the cut. Acid ammonium urate may exist in urine which has become alkaline from the decomposition of urea and formation of ammonium carbonate, and may therefore be seen in company with the phosphate sediments. The other urates dissolve in alkaline urine. Like uric acid the urates appear in a great variety of forms, and there is still some uncertainty about the composition of some of their crystals which have been found in urine.

### Leucin and Tyrosin

These two substances are of rare occurrence in urine and appear only under pathological conditions. Urine containing them shows usually strong indications of the presence of biliary matters as they generally are found in consequence of some grave disorder of the liver in which destruction of its tissue is involved. They have been most frequently found, and associated, in acute yellow atrophy of the liver and in severe cases of phosphorus poisoning. In general they must be considered as products of disintegration and are produced in the intestine in large quantity by bacterial agency in the last stages of the digestion of albuminoids, as was pointed out in an earlier chapter.

As both bodies are slightly soluble they may not be seen directly, but only after partial concentration of the urine. In pure condition leucin crystallizes in thin plates but from urine it separates in spherical

bunches made up of fine plates or needles. These bunches are sometimes so compact that it is hard to distinguish between them and other substances, particularly lime soaps and oil drops. Chemical tests must therefore be applied. If mercurous nitrate is added to a leucin solution and the mixture is warmed, metallic mercury precipitates. This test can be car-

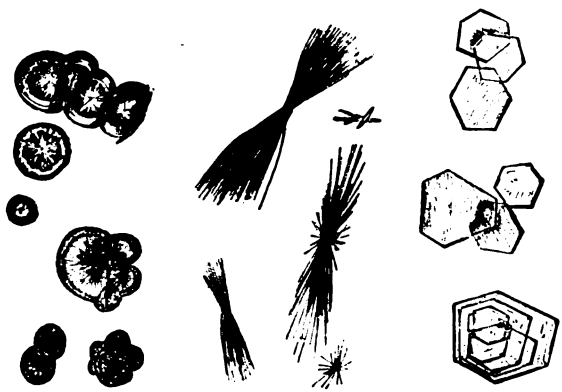

Fig. 24. Leucin spheres, tyrosin needles and cystin plates.

ried out only when the substance is abundant enough to be purified by crystallization from hot water. Pure leucin, when strongly heated with nitric acid on platinum, forms a colorless residue, which when heated with potassium hydroxide leaves an oil-like drop that does not wet the platinum.

Tyrosin is usually seen in long needles, which sometimes are bunched in the form of sheaves, and is more readily recognized than is leucin. Tyrosin heated with nitric acid on platinum turns orange-yellow, and leaves a dark residue which becomes reddish yellow

by addition of caustic alkali. Solutions containing tyrosin, when treated hot with mercuric nitrate and potassium nitrite, turn red and finally throw down a red precipitate.

Leucin and tyrosin may be present in the urine yet not show as a sediment. For their detection under these circumstances precipitate the urine with basic lead acetate and filter. Separate the excess of lead from the filtrate by hydrogen sulphide and filter again. Concentrate the filtrate on a water-bath to a sirup and treat it with a little *absolute* alcohol to remove urea. Some leucin may dissolve with the urea in this treatment. Next boil the residue with 60 or 70 per cent. alcohol, filter, concentrate the filtrate to a small volume and allow it to stand in a cool place for crystallization. If crystals appear their form indicates whether they consist of pure tyrosin or a mixture of tyrosin and leucin. The latter, being more soluble in strong alcohol, can be separated by washing with this liquid. The final tyrosin residue can be used for the tests given above.

The alcoholic solutions may contain leucin, which can be recognized after evaporation. Both leucin and tyrosin decompose readily in urine undergoing putrefactive changes; it is therefore necessary to apply the test to urine as fresh as possible.

## Cystin

This is a rare sediment, although it is found constantly in the urine of certain individuals. It crystallizes in thin hexagonal plates, small ones sometimes

resting upon or overlapping large ones. The crystals are regular in form but variable in size and readily recognized. A rare form of uric acid crystallizes in a somewhat similar manner but the two substances differ in their behavior toward ammonia. To distinguish between them in the microscopic test place a drop of ammonia water on the slide and allow it to pass under the cover glass. Cystin dissolves, but, unless heated, uric acid does not. When the ammonia evaporates cystin reprecipitates.

Cystin is precipitated from urine by addition of acetic acid. Mucin and uric acid may come down at

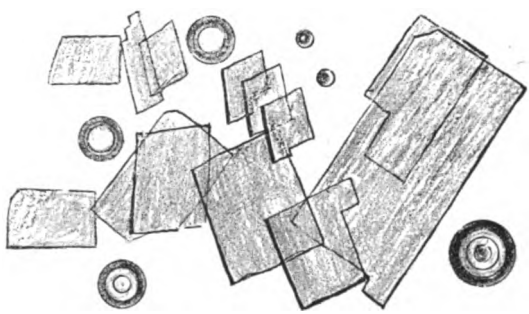

Fig. 25. Cholesterin plates and fat globules.

the same time. The precipitate is collected on a filter, washed with water and finally dissolved in ammonia. By neutralizing the ammoniacal filtrate with acetic acid and concentrating a little, it comes down in the characteristic form suitable for microscopic recognition.

## CHOLESTERIN

This substance occurs occasionally in urine, and has been found in cases of cystitis and chyluria. It is

recognized by its characteristic crystalline form, large, thin plates, shown in the cut (Fig. 25). These plates are nearly transparent but from their size cannot be overlooked. Cholesterin is a common constituent of biliary calculi.

### Fat Globules

These are often seen in urine, but in most cases have not been voided with it. They can come from several extraneous sources, as from a catheter, from vessels in which the urine is collected or sent for examination,

Fig. 26. Hippuric acid.

from admixed sputum, etc., which facts should be borne in mind.

Fat has been found in cases of fatty degeneration of the kidney and more abundantly in chyluria where communication seems to be formed between the lymphatics and the urinary tract by the invasion of the small thread worms referred to above.

### Hippuric Acid

This acid is found normally in human urine in small amount. It may be found in large quantity after taking benzoic acid and may even appear in crystalline form in the sediment. It has no pathological importance, ordinarily.

### Calcium Carbonate

This is sometimes observed as a coarse, granular sediment which dissolves with effervescence in acetic acid. It occasionally forms dumb-bell crystals, and is devoid of pathological importance.

### Calcium Sulphate

Crystals of this substance are rarely found in urine. They form long, colorless needles, or narrow, thin plates.

### Calcium Oxalate

We have here one of the commoner of the crystalline bodies observed in urine.

This may be found in neutral or alkaline urine, but more commonly in that of acid reaction. It occurs normally and sometimes is very abundant, especially after the consumption of vegetables containing oxalic acid.

Two principal forms of the crystals are found, the octahedral and dumb-bell crystals.

The octahedra have one very short axis which gives the crystals a flat appearance. When seen with the short axis perpendicular to the plane of the cover glass, which is the common position, they appear as

squares crossed by two bright lines. Sometimes they are seen on edge, and then present a rhomb in section with one diameter very much shorter than the other.

A form of triple phosphate bears a slight resemblance to calcium oxalate, but it is soluble in acetic acid, while the oxalate is not.

The dumb-bells are much less common than the octahedra, and are found in several modified forms, as shown in one of the figures.

The clinical significance of the oxalate is not clearly

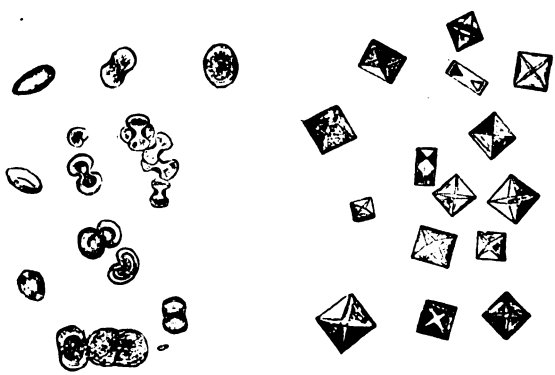

Fig. 27. Calcium oxalate.

understood. It does not seem to be characteristic of any disease even when occurring in quantity. It has been found considerably increased in dyspeptic conditions, but not always, and many of the statements found concerning its significance seem to have been based on insufficient observations.

Urine may contain a large amount of oxalic acid which does not show as a sediment, but must be found

by precipitation with calcium chloride in presence of ammonium hydroxide. Acetic acid is then added in very slight excess and the mixture is allowed to stand for precipitation.

The constant or prolonged excretion of large amounts of oxalic acid is spoken of as oxaluria.

## The Phosphates

It was explained in Chapter VII that phosphates of alkali and alkali-earth metals occur normally in the urine, and a method was given for their estimation. As sediment we know several forms of calcium and

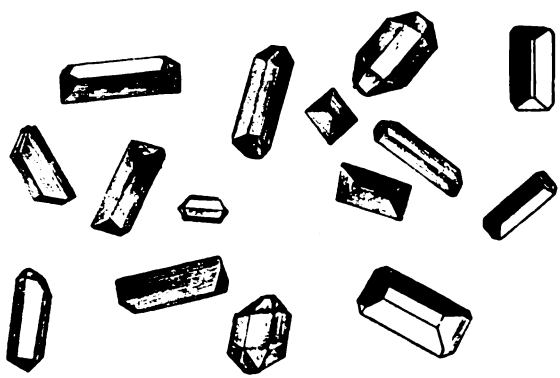

Fig. 28. Triple phosphate.

magnesium phosphates and the microscopic detection of these will be here explained. In normal fresh urine of acid reaction these phosphates are held in solution, but if the urine as passed is alkaline it is often turbid from the presence of basic phosphates held in suspension. Urine which has stood long enough to undergo the alkaline fermentation always contains phosphates

in the sediment. Finally, it must be remembered that a neutral or very slightly acid urine, containing ammonium salts in abundance, may also deposit a crystalline precipitate of ammonium magnesium phosphate. The common phosphate sediments are those consisting of ammonium magnesium phosphate (triple phosphate), normal magnesium phosphate, neutral cal-

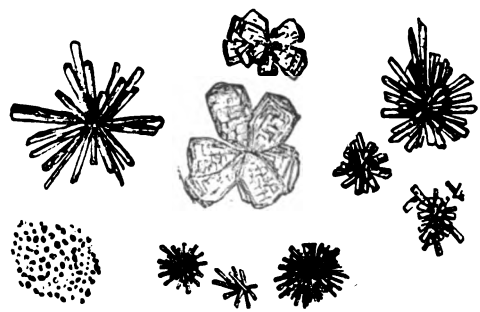

Fig. 29. Neutral calcium phosphate and amorphous phosphate.

cium phosphate, and mixed amorphous phosphates of calcium and magnesium.

**Triple Phosphate.**—Of the crystalline phosphate deposits this is the most abundant and at the same time the most characteristic.

The crystals are the largest found in urine, and from their shape are sometimes spoken of as coffin-lid crystals. Ordinarily they are not found in perfectly fresh urine, but after it has undergone the alkaline fermentation they are generally present in profusion.

**Normal Magnesium Phosphate.**—Crystals having the composition, $Mg_3(PO_4)_2.22H_2O$, are sometimes found in urine of nearly neutral reaction. They consist of

thin, transparent, rhombic plates with angles of approximately 60° and 120°. If urine containing this sediment becomes alkaline, triple phosphate forms.

**Neutral Calcium Phosphate.**—This has the composition, $CaHPO_4.2H_2O$, and is found in urine of neutral or slightly acid reaction. It crystallizes frequently in rosettes formed of wedge-shaped, single crystals, uniting at their apices. The cut shows some variations in the form.

**Amorphous Phosphates.** —Finally we have the very common, finely granular, earthy phosphates in amorphous condition. This sediment dissolves readily in weak acetic acid and is colorless. The common amorphous urate sediment is colored and does not dissolve in acetic acid. On addition of sodium carbonate or hydroxide to urine, the precipitate which forms consists mainly of this phosphate.

These several phosphates can be produced artificially and should be made for study and comparison. The normal magnesium phosphate can be made by dissolving 15 grams of crystallized common sodium phosphate in 200 cc. of water and mixing this with 3.7 grams of crystallized magnesium sulphate in 2000 cc. of water. Enough sodium bicarbonate is added to give an amphoteric reaction and then the mixture is allowed to stand a day or more for precipitation.

Crystals of triple phosphate of peculiar form are often obtained by adding ammonia to urine, and sometimes a trace of ammonia is sufficient to throw down the crystals of neutral calcium phosphate. The latter

can also be obtained by adding to a weak solution of crystallized sodium phosphate a trace of acid and then a very little calcium chloride solution.

### Foreign Matters

The sediment of urine often contains foreign substances which have become mixed with it accidentally. The most common of these are hairs, woolen, cotton or silk fibers, granules of starch, fat globules, dust and

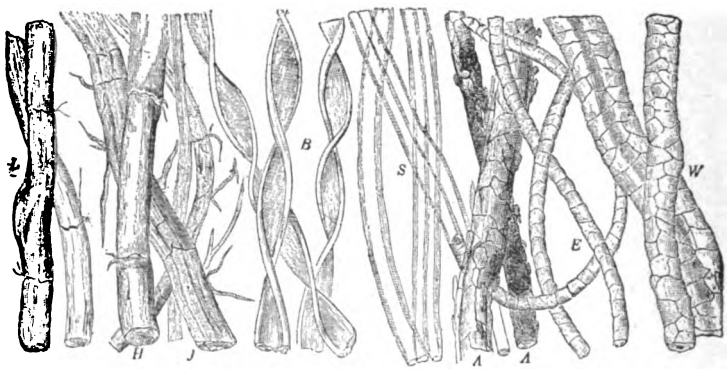


Fig. 30. L, linen fibers; H, hemp fibers; J, jute fibers; B, cotton fibers; S, silk fibers; A, alpaca fibers; E, fine wool; W, common wool.

sand granules, bits of woody fiber and remains of articles of food. Some of these are represented in Figs. 30 and 31.

### Urinary Calculi

Calculi, like the sediments just described, are formed by the precipitation of certain substances from the urine, but in compact form. Occasionally a calculus

consists of a single substance, as calcium oxalate or cystin, but in the great majority of cases a mixture of bodies is present, these being deposited usually in layers around a nucleus which serves as the foundation of the concretion. Calculi are built up much as certain forms of crystals are by successive depositions on a nucleus. Uric acid is a very common nucleus on which may be deposited urates, phosphates, organic matters, etc.

Calculi are sometimes distinguished as *primary* or *secondary*. Primary calculi may be traced to an alter-

Fig. 31. Left, pubic hair with spermatozoa; center, hair of woman's head; right, cat's fur.

ation of the urine of such a nature that its reaction is constantly acid. The foundation for the concretions in this case is found in the kidney and they are built up of such substances as most easily deposit from acid urine. Secondary calculi are generally formed in the bladder, and have for nuclei matters precipitated from alkaline urine, as coagulated blood or other organic substances. Sometimes fragments introduced into the bladder from without serve as the foundation for these

secondary formations. Bits of catheters, remains of bougies, and other things have been found as the nuclei around which concretions have formed. The recognition of the nucleus is a matter of the first importance as this gives a clew to the determining cause active in the formation of the calculus.

In making an examination, then, of a calculus, it is first cut in two by means of a very sharp thin saw. This exposes the nucleus which may often be recognized by the eye alone. If one of the halves be polished it is often possible to discern distinctly the various layers grouped around the center.

In a large number of cases examined by Ultzmann about 80 per cent. were found to contain uric acid as the nucleus.

### Chemical Examination

In the chemical examination of a calculus several methods may be employed. We may begin by applying certain preliminary tests designed to show the general nature of the stone.

**Heat Test.**—Reduce some of the calculus to a powder and heat to bright redness on platinum foil. Two cases may arise: (*a*), the powder is completely consumed; (*b*), the powder is only partially consumed or not at all.

*Case* (*a*). If this is the result of the incineration the following substances may be suspected:

**Uric Acid,** which may be recognized by dissolving a little of the powder in weak alkali, precipitating by

hydrochloric acid and examining the precipitate by the microscope.

**Ammonium Urate.**—This gives the above reaction under the microscope, and is further recognized by the liberation of ammonia when heated with a little pure sodium hydroxide solution.

**Cystin.**—Dissolve some of the powder in ammonia, filter if necessary and allow drops of the filtrate to evaporate spontaneously on a slide. Cystin is then recognized by the microscope as already explained. Cystin contains sulphur which, on burning on the platinum foil, gives rise to a disagreeable sharp odor. If a little of the powder be heated with a mixture of potassium nitrate and sodium carbonate the sulphur is oxidized to sulphate, which may be recognized by the usual tests.

**Xanthin.**—This is a rare substance in calculi. Those consisting wholly of xanthin are brown in color and take a wax-like polish. A method of recognition will be given below.

**Organized Matter.**—Parts of blood cells, epithelium, precipitated mucin, pus corpuscles and similar substances may become entangled with the growing stone and even form a large part of it. On burning, these bodies are recognized by the characteristic odor of nitrogenous matter.

*Case* (*b*). When an incombustible residue is left on the platinum foil the stone may contain the following constituents:

**Calcium Oxalate.**—Stones of this substance are very

hard and break with a crystalline fracture. They are often called "mulberry calculi." When the powder is heated it decomposes, leaving carbonate, which may be recognized by its effervescence with acids.

**Calcium and Magnesium Phosphates.**—They leave a residue in which the metals and phosphoric acid may be detected by simple tests of qualitative analysis. The ignited powder is soluble in hydrochloric acid without effervescence. When ammonia is added to this solution in quantity sufficient to give an alkaline reaction, a precipitate of triple phosphate or calcium phosphate appears, which may be recognized by the microscope.

The above tests are generally sufficient to tell all that is practically necessary about the calculus. If more detailed information is desired a systematic analysis may be made according to the following scheme :

**Systematic Analysis.**—1. Reduce the calculus to a fine powder and pour over it some water and finally dilute hydrochloric acid in a beaker. Warm gently half an hour, or longer, on the water-bath. Then allow to cool and filter.

2. Treatment of the residue. It seldom happens that the calculus is completely soluble in the weak acid. A residue usually remains which may contain uric acid, xanthin, calcium sulphate, and remains of organized matter. To prove the xanthin treat the residue with warm *dilute* ammonia and filter. The filtrate contains the xanthin if it is present. Acidify

it with nitric acid and add a small amount of silver nitrate solution. This produces a flocculent precipitate which dissolves by warming, and crystallizes on cooling in bunches of fine needles.

In the residue free from the xanthin look for calcium sulphate by extracting with water and applying the usual tests. This solution may contain uric acid which is recognized by evaporation and crystallization after adding a little hydrochloric acid. In the final residue some uric acid may be also present. Dissolve in alkali, reprecipitate with hydrochloric acid, and examine any crystals which may form under the microscope.

3. Treatment of the hydrochloric acid solution. This may contain calcium oxalate, cystin, the phosphates, and possibly some xanthin. Look for the last in a small portion of the solution. Make this portion alkaline with ammonia, add a few drops of calcium chloride solution, filter if a precipitate forms, and treat the filtrate with ammoniacal silver nitrate solution. In presence of xanthin a flocculent precipitate forms.

Dilute the remaining and larger portion of the hydrochloric acid solution with twice its volume of water, add enough ammonia to give a strong alkaline reaction, and then acetic acid to restore a weak acid reaction. By this treatment phosphates are held in solution while calcium oxalate, if present, precipitates. Therefore allow the mixture to stand half an hour and then filter off any precipitate which appears. This precipitate may contain cystin as well as calcium oxalate. Cystin may be dissolved by pouring ammonia on the

filter, and on evaporating the ammoniacal solution is obtained in form suitable for microscopic examination.

The residue free from cystin is dried and heated to redness on platinum foil. This treatment converts calcium oxalate into carbonate. Place the foil in a beaker and add some dilute acetic acid; an effervescence shows the carbonate. To the clear solution add next some ammonium oxalate which gives a white precipitate of calcium oxalate, if the latter metal is present.

We have next to look for the phosphates and bases in the acetic acid solution obtained after filtering off cystin and calcium oxalate. More calcium may be present, in excess of that combined as oxalate, which may be recognized by adding a little solution of ammonium oxalate. If a precipitate forms treat the whole of the liquid with the ammonium oxalate; after warming on the water-bath, allow to stand an hour and filter. Concentrate the filtrate in platinum to a small volume, transfer to a large test-tube and add enough ammonia to produce an alkaline reaction. If a precipitate appears now it must consist of magnesium phosphate, showing both magnesium and phosphoric acid as present in the original. If no precipitate appears magnesium is absent but phosphoric acid may still be present. To find it divide the ammoniacal liquid into two portions. To one add a few drops of magnesia mixture, and to the other add nitric acid in slight excess and then ammonium molybdate reagent. Both tests should yield the reactions characteristic of phosphates, if present.

This procedure serves for the recognition of the important constituents of calculi. But ammonium, potassium, and sodium compounds are sometimes present and may be recognized readily. To detect ammonium salts the original calculus powder may be heated with pure potassium hydroxide solution, or the hydrochloric acid solution of the calculus may be neutralized and heated with the same solution. The ammonia is recognized by the odor or by its reaction on moistened red litmus paper.

To recognize the alkali metals a solution of the powdered calculus in hydrochloric acid is treated with pure ammonia and a little ammonium carbonate in excess. The precipitate formed is allowed to settle and filtered off. The filtrate is then evaporated to dryness in a platinum dish and the residue strongly heated to drive off all ammonium salts. What is now left contains sodium and potassium if they were present in the original. Moisten this final residue with water and a drop of hydrochloric acid and test with a platinum wire in the flame of a Bunsen burner, using a deep blue glass when looking for potassium. Only a very intense yellow color can be taken as indicative of sodium.

# APPENDIX

## Tables and Notes

### Tables of Weights and Measures

THE METRIC SYSTEM

1 meter = 100 centimeters, cm. = 1000 millimeters, mm.
1 liter = 1000 cubic centimeters, cc.
1 kilogram = 1000 grams, gm.
1 gram = 1000 milligrams, mg.

AMERICAN WEIGHTS AND MEASURES

1 gallon = 8 pints.
1 pint = 16 fluidounces.
1 fluidounce = 8 fluidrachms.
1 fluidrachm = 60 minims.
1 avoirdupois pound = 16 avoirdupois ounces.
1 avoirdupois ounce = 437½ grains.
1 apothecaries' ounce = 8 drachms.
1 apothecaries' drachm = 60 grains.

EQUIVALENTS

1 meter = 39.370 inches.
1 liter = 33.815 U. S. fluidounces.
1 liter = 35.219 Imp. fluidounces.
1 cubic centimeter = 16.231 U. S. minims.
1 kilogram = 32.151 U. S. apoth. ounces.
1 kilogram = 35.274 avoirdupois ounces.
1 gram = 15.432 grains.
1 U. S. fluidounce = 29.57 cubic centimeters.
1 Imp. fluidounce = 28.39 cubic centimeters.
1 U. S. apoth. ounce = 31.103 grams.
1 avoirdupois ounce = 28.349 grams.
1 grain = 64.798 milligrams.

1 U. S. fluidounce of water weighs 0.95 U. S. apoth. ounce.
96 U. S. fluidounces of water weigh 100 avoirdupois ounces.
1 Imperial fluidounce of water weighs 1 avoirdupois ounce.

1 U. S. gallon = 3.785 liters.
1 avoird. pound = 453.59 grams.
1 kilogram = 2.204 avoir. pounds.
1 pint = 473.179 cubic centimeters.

## Table of Approximate Atomic Weights

(O = 16 as basis)

| Name. | Symbol | Atomic Weight | Name. | Symbol | Atomic Weight |
|---|---|---|---|---|---|
| Aluminum. . . . | Al | 27.1 | Magnesium . . | Mg | 24.3 |
| Antimony. . . . | Sb | 120.4 | Manganese. . . | Mn | 55.0 |
| Arsenic. . . . . | As | 75.0 | Mercury . . . . | Hg | 200.0 |
| Barium . . . . . | Ba | 137.4 | Molybdenum. . | Mo | 96.0 |
| Bismuth . . . . . | Bi | 208.1 | Nickel . . . . . | Ni | 58.7 |
| Boron . . . . . | B | 11.0 | Nitrogen . . . . | N | 14.0 |
| Bromine . . . . . | Br | 80.0 | Oxygen . . . . | O | 16.0 |
| Cadmium . . . . | Cd | 111.9 | Phosphorus . . | P | 31.0 |
| Calcium . . . . . | Ca | 40.1 | Platinum. . . . | Pt | 194.9 |
| Carbon. . . . . | C | 12.0 | Potassium . . . | K | 39.1 |
| Chlorine . . . . . | Cl | 35.5 | Selenium . . . | Se | 79.0 |
| Chromium . . . | Cr | 52.1 | Silicon . . . . . | Si | 28.4 |
| Cobalt. . . . . | Co | 58.9 | Silver . . . . . | Ag | 107.9 |
| Copper . . . . . | Cu | 63.6 | Sodium . . . . | Na | 23.0 |
| Fluorine . . . . . | F | 19.0 | Strontium . . . | Sr | 87.6 |
| Gold . . . . . | Au | 197.2 | Sulphur . . . . | S | 32.1 |
| Hydrogen . . . . | H | 1.0 | Tellurium . . . | Te | 127.5 |
| Iodine . . . . . | I | 126.9 | Thallium . . . . | Th | 204.1 |
| Iron . . . . . | Fe | 56.0 | Tin . . . . . | Sn | 119.0 |
| Lead . . . . . | Pb | 206.9 | Uranium . . . . | U | 239.6 |
| Lithium . . . . | Li | 7.0 | Zinc . . . . . | Zn | 65.4 |

## Volume and Specific Gravity of Water of Different Temperatures

Calculations of Volkmann from results of Kopp, Hagen, Matthiessen, Jolly, and Pierre.

| Temperature C. | Specific gravity, or weight of 1 cc. of water in vacuo in grams. | Differences for 1°, sp. gr. and volume | Volumes of one gram of water in cc. |
|---|---|---|---|
| 0 | 0.99988 | ——— | 1.00012 |
| 1 | 0.99993 | 0.00005 | 1.00007 |
| 2 | 0.99997 | 0.00004 | 1.00003 |
| 3 | 0.99999 | 0.00002 | 1.00001 |
| 4 | 1.00000 | 0.00001 | 1.00000 |
| 5 | 0.99999 | 0.00001 | 1.00001 |
| 6 | 0.99997 | 0.00002 | 1.00003 |
| 7 | 0.99993 | 0.00004 | 1.00007 |
| 8 | 0.99988 | 0.00005 | 1.00012 |
| 9 | 0.99982 | 0.00006 | 1.00018 |
| 10 | 0.99974 | 0.00008 | 1.00026 |
| 11 | 0.99965 | 0.00009 | 1.00035 |
| 12 | 0.99954 | 0.00011 | 1.00046 |
| 13 | 0.99943 | 0.00011 | 1.00057 |
| 14 | 0.99930 | 0.00013 | 1.00070 |
| 15 | 0.99915 | 0.00015 | 1.00085 |
| 16 | 0.99900 | 0.00015 | 1.00100 |
| 17 | 0.99884 | 0.00016 | 1.00116 |
| 18 | 0.99866 | 0.00018 | 1.00134 |
| 19 | 0.99847 | 0.00019 | 1.00153 |
| 20 | 0.99827 | 0.00020 | 1.00173 |
| 21 | 0.99807 | 0.00021 | 1.00194 |
| 22 | 0.99785 | 0.00022 | 1.00216 |
| 23 | 0.99762 | 0.00023 | 1.00238 |
| 24 | 0.99739 | 0.00023 | 1.00262 |
| 25 | 0.99714 | 0.00025 | 1.00287 |
| 30 | 0.99577 | 0.00027 | 1.00425 |

## List of General Reagents and Test Solutions

**Acid, sulphuric (strong).**—The commercial acid is sufficient for most purposes, where a strong acid is called for. Where the pure acid is required it is mentioned in the text.

**Acid, sulphuric (dilute).**—Add 1 part of the above acid to 4 parts of distilled water, mix thoroughly, and allow to stand twenty-four hours. Then siphon, or pour off the clear liquid, which is ready for use. The strong acid must be poured into the water very slowly, and with constant stirring, to avoid a too sudden elevation of temperature.

**Acid, nitric (strong).**—The strong commercial acid can be employed in most cases where this acid is called for. A pure strong acid is also employed, occasionally.

**Acid, nitric (dilute).**—Where this acid is called for as a reagent it should be made by mixing 1 part of the *pure* strong acid with 4 parts of distilled water. It should be free from traces of chlorine and sulphates.

**Acid, hydrochloric (strong).**—The yellow commercial acid is largely used in the laboratory in the preparation of other substances. It is seldom pure enough to be employed as a test reagent.

A colorless acid, free from organic matter, iron and traces of sulphates, must be used when the pure strong acid is called for.

**Acid, hydrochloric (dilute).**—This is frequently used in liberating hydrogen sulphide, carbon dioxide, and hydrogen, and for other purposes, and need not be pure. Where the dilute acid is called for as a reagent it must be made by mixing 1 part of the pure strong acid with 4 parts of distilled water.

**Acid, acetic.**—Mix 1 part of the pure "glacial" acid with 4 parts of water.

**Ammonia water.**—The strong solution is seldom used in analysis. The solution usually employed is made by mixing 1 volume of the stronger ammonia water (containing about 28 per cent. of the gas) with 3 volumes of distilled water.

The solution should be free from carbonic acid, as presence of this would interfere with several of the tests where it is employed.

**Ammonium carbonate.**—Dissolve 1 part of the pure powdered crystals in 5 parts of dilute ammonia water.

**Ammonium chloride.**—Dissolve 1 part of the pure salt in 10 parts of water.

**Ammonium molybdate.**—A solution of this salt is chiefly used as a test for phosphoric acid, and should be prepared in this way: Dissolve 3 grams of the crystals in 20 cc. of water, and pour this solution in 20 cc. of strong nitric acid. Warm the mixture to about 40° C. (not above), and allow to settle.

As the reagent does not keep well, it should be made only in small quantities.

**Ammonium oxalate.**—Dissolve 1 part of the pure crystals in 20 parts of water.

**Ammonium sulphide.**—Dilute a quantity of strong ammonia water with an equal volume of water. Take three-fifths of this mixture and saturate it with hydrogen sulphide. Then add the remaining two-fifths of the diluted ammonia water and mix thoroughly.

**Barium chloride.**—Dissolve 1 part of the crystals in 10 parts of water.

**Calcium hydroxide.**—Slake pure white lime and pour over it a large excess of water. Allow to settle and throw away the clear liquid. Again add pure water, shake thoroughly, allow to settle as before, and decant the clear liquid into bottles for use. These bottles must be tightly stoppered. The portion rejected contains small amounts of impurities, possibly present in the lime.

**Lead acetate.**—One part of the pure crystals to 10 parts of water. It may be necessary to add a few drops of acetic acid to secure a clear solution.

**Lead acetate, basic.**—To make a liter of solution of this reagent weigh out 170 grams of lead acetate and 120 grams of yellow lead oxide.

Dissolve the lead acetate in 800 cc. of boiling, distilled water, in a glass or porcelain vessel. Then add the oxide of lead and boil for half an hour, occasionally adding enough hot distilled water to make up the loss by evaporation. Remove the heat, allow the liquid to cool, and add enough distilled water, previously boiled and cooled, to make the product measure 1000 cc. Finally, filter the liquid in a covered funnel.

Solution of basic lead acetate should be kept in well-stoppered bottles.

**Magnesia mixture.**—Dissolve 100 grams of magnesium sulphate and 100 grams of ammonium chloride in 800 cc. of water, and add 100 cc. of strong ammonia water. Allow the mixture to stand twenty-four hours, and filter.

**Mercuric chloride.**—One part of the pure crystals to 20 parts of water.

**Millon's reagent.**—Dissolve 1 part of mercury in 2 parts of strong nitric acid, by aid of heat finally, and after cooling dilute the solution with twice its volume of water.

**Potassium chromate.**—Dissolve 1 part of the crystals, free from chlorine, in 10 parts of water.

**Potassium dichromate.**—Dissolve 1 part of the pure crystals in 10 parts of water. The dry powdered crystals are also used.

**Potassium ferrocyanide.**—Dissolve 1 part of the crystals in 20 parts of water.

**Potassium hydroxide.**—Several solutions are employed in analytical chemistry. For most purposes one containing 10 per cent. of the "stick" hydroxide is sufficient.

**Sodium hydroxide.**—Dissolve 1 part of the best "stick" hydroxide in 10 parts of water, allow to settle, and decant the clear solution.

This solution acts on glass bottles, and soon deposits a sediment. Hence, a great deal of it should not be made at one time. The glass stoppers of bottles containing sodium hydroxide, and many other substances, should be covered

with a thin layer of paraffin, which prevents their sticking fast.

**Sodium hypochlorite.**—The "chlorinated soda" of the U. S. P. is to be used here, and the solution may be made in this manner: Weigh out 75 grams of good commercial "chloride of lime" and rub it up with 200 cc. of water to a thin cream. Allow this to settle and pour the liquid through a filter. Stir the residue with a second 200 cc. of water, pour the whole on the filter and wash the insoluble residue with 100 cc. of water, allowing this to mix with the 400 cc. Now dissolve 150 grams of sodium carbonate crystals in 300 cc. of hot water and pour this into the other solution. Warm the mixed solution and stir it well. Pour the mixture on a filter and when the liquid has run through pour on water enough to bring the filtrate up to 1000 cc. The solution so obtained should be kept in the dark.

## Special Reagents

### Solutions for Sugar Tests

**Fehling solution.**—This has been referred to at length in Chapter III, and its preparation will now be given. In presence of alkali, copper solutions are reduced by dextrose approximately according to this proportion:

$$\underset{1248.00}{5(CuSO_4 \cdot 5H_2O)} : \underset{180}{C_6H_{12}O_6}.$$

from which it follows that 34.66 mg. of the crystallized sulphate in solution oxidizes 5 mg. of dextrose in solution.

The Fehling solution proper consists of a mixture of copper sulphate, an alkali, and a tartrate. Investigation has shown that alkali sodium hydroxide is preferable and that Rochelle salt is the best tartrate for the purpose. It has also been found that the best results are obtained if the copper and tartrate are mixed just before needed for use. Therefore, prepare solutions separately as follows:

1. Dissolve 69.32 grams of pure, recrystallized copper sulphate in distilled water to make 1 liter of solution. Much of

the copper sulphate sold by druggists contains ferrous sulphate and is not suitable for the purpose.

2. Dissolve 100 grams of sodium hydroxide in sticks in 500 cc. of water, heat to boiling and add gradually 350 grams of pure recrystallized Rochelle salt. Stir until all is dissolved. Allow the solution to stand twenty-four hours in a covered vessel, then filter through asbestos into a liter flask and add water enough to make the solution 1 liter. The sodium hydroxide for this purpose should be the grade designated "precipitated by alcohol" and the Rochelle salt should be practically pure.

3. By mixing equal volumes of these two solutions the Fehling liquid is prepared, containing 34.66 mg. of the copper sulphate in each cubic centimeter. This mixture is made when required for use.

**The Loewe solution.**—According to Loewe, copper solutions prepared with glycerol are more stable than are those with a tartrate, while the oxidizing power of the copper hydroxide is practically the same. For quantitative tests Loewe prepares a solution by mixing

| | |
|---|---|
| Crystallized copper sulphate . . | . 34.66 gm. |
| Pure glycerol . . . . | 26.00 gm. |
| Sodium hydroxide solution, 1.34 sp. gr. | . 70.00 cc. |

with a small amount of water and heating to dissolve, after which the solution is diluted to 1 liter.

When used as a qualitative test this solution is diluted by adding glycerol. In place of the crystallized copper sulphate Loewe recommends to weigh out the corresponding amount of precipitated, washed, and dried copper hydroxide, which can be kept indefinitely in perfectly stable form. Haines' solution is a modification of the Loewe solution containing a relatively large amount of glycerol and alkali, and is employed in qualitative testing.

**Pavy's solution.**—The use of the Pavy liquid as a sugar test was explained in Chapter III. It is prepared as follows:

Dissolve 34.66 grams of crystallized copper sulphate, 170

grams of Rochelle salt, and 170 grams of good stick potassium hydroxide in distilled water to make 1 liter. Mix 120 cc. of this solution with 400 cc. of strong ammonia water, sp. gr. 0.88, and dilute with distilled water to 1 liter. The oxidizing power of this solution is assumed to be just one-tenth of that of the Fehling solution, which would follow if the reaction takes place in the proportion $6(CuSO_4.5H_2O):C_6H_{12}O_6$.

**Loewe-Pavy solution,** as recommended by Dr. Purdy.

This is made by dissolving

| | |
|---|---|
| Crystallized copper sulphate . . . | 4.75 gm. |
| Potassium hydroxide . . . . . | 23.50 gm. |
| Ammonia water, sp. gr. 0.90. . . | 350.00 cc. |
| Glycerol . . . . . | 38.00 cc. |

in enough water to make 1000 cc.

Dissolve the copper sulphate and glycerol in 200 cc. of the water. In another 200 cc. dissolve the alkali. Mix the two solutions, cool, add the ammonia and make up to 1000 cc. Pure chemicals must be employed.

According to Dr. Purdy 35 cc. of this solution oxidize 20 mg. of dextrose. But the oxidizing power of the solution depends on the amount of ammonia and fixed alkali present and on the concentration of the sugar solution.

In the Pavy and Purdy liquids the oxidizing power is apparently assumed to be independent of the strength of the sugar solution added. Pavy and Purdy assume the factor 8.316 $CuSO_4.5H_2O$ to 1 $C_6H_{12}O_6$. But Peska has shown that the factor varies over 2 per cent with solutions ranging from 0.1 to 1 per cent in strength.

As in practical work it is desirable to employ a solution, 1 cc. of which oxidizes some simple unit amount of sugar, the author has been using lately one with a value of 1 cc. for each milligram of sugar oxidized in 0.2 per cent. solutions. It is made with the following amounts per liter:

| | |
|---|---|
| Copper sulphate, cryst - - - | 8.166 gm. |
| Sodium hydroxide (100 per cent.) - | 15.000 gm. |
| Glycerol - - - - - - | 25.000 cc. |
| Ammonia water, 0.9 sp. gr., - - | 350.000 cc. |
| Water to make - - - - | 1,000.000 cc. |

Of this solution use 50 cc. and dilute with water to 100 cc. To prevent too rapid an escape of ammonia and avoid reoxidation to some extent, add to the mixture, while warming, enough pure white solid paraffin to make a layer 3 or 4 millimeters in thickness when melted. The burette tip for discharging the sugar solution or urine is made long enough to pass down the neck of the flask and below this paraffin. By boiling gently and adding the weak saccharine liquid slowly, very close and constant results may be obtained. At the end of the titration the paraffin is solidified by inclining the flask and immersing it in cold water, or by flowing cold water over it. The reduced liquid is then poured out and the cake of paraffin is thoroughly washed for the next test. A flask so prepared may be used for a hundred titrations. The solid paraffin is much preferable to the oil recommended by Allen and Peska. To prevent bumping and facilitate easy and uniform boiling, it is well to add a few very small fragments of pumice-stone. A solution made as above is not too strong in copper for accurate work, but the volume of ammonia necessary to hold a much larger amount of the reduced oxide in solution would render the process very inconvenient.

**Schmiedeberg's solution.**—Dissolve 34.66 grams of pure crystallized copper sulphate in 200 cc. of water and 16 grams of mannitol in 100 cc. of water. Mix the two solutions and add 480 cc. of sodium hydroxide solution, having a specific gravity of 1.145. Dilute to 1 liter. This solution is assumed to have the oxidizing power of the Fehling solution.

**Knapp's solution.**—This is made by dissolving 10 grams of dry mercuric cyanide in 600 cc. of distilled water. To this solution is added 100 cc. of a solution of sodium hydroxide, having a specific gravity of 1.145, and the mixture is diluted to 1 liter. 10 cc. of this solution may be taken as sufficient to oxidize 25 mg. of dextrose in dilute solution. In strong solution the oxidizing power is less. The test should be made with a dilution as indicated in the text.

**Sachsse's solution.**—Dissolve 18 grams of pure mercuric iodide and 25 grams of potassium iodide in water. Add a solution of 80 grams of good potassium hydroxide and dilute to 1 liter. It has been shown that 50 cc. of Sachsse's solution is reduced by 168 mg. of dextrose, in a solution of about 1 per cent. strength.

**Fron's reagent.**—Dissolve 7 grams of potassium iodide in 20 cc. of water; heat and add 1.5 grams of bismuth subnitrate and 1 cc. of strong pure hydrochloric acid.

## Indicators

The indicators most commonly employed in the titration of acids and alkalies are aqueous or alcoholic solutions of *litmus*, *cochineal*, *phenol-phthalein*, *methyl orange*, and *rosolic acid*. In addition to these, certain others are employed for special purposes, and among them there may be mentioned, *congo red*, *benzopurpurin*, *methyl violet*, and the *tropæolins*. A few explanations will be given on the preparation of the first.

**Litmus.**—Crude litmus comes in commerce in the form of small blue cubes. These are powdered and extracted by hot water. The aqueous solution is concentrated and acidified with acetic acid, after which it is evaporated to a paste. Treat this with an excess of 85 per cent. alcohol which dissolves foreign matters but leaves the true color. Throw the mixture on a filter and wash the residue with strong alcohol. Then dissolve the precipitated color on the filter by means of hot water and keep this aqueous solution for use in a bottle loosely stoppered, as access of air is necessary for its preservation.

A neutral litmus solution is used for certain purposes and may be prepared by dividing the aqueous solution just described into two portions, one of which is rendered faintly acid by nitric acid, while the other is made alkaline by potassium hydroxide, added in drops of very dilute solution. On mixing these two liquids the product will be found practically neutral.

**Cochineal.**—A solution is made by extracting 1 part of the crushed cochineal with 10 parts of weak alcohol. This indicator is valuable in titrating in presence of carbonic acid or ammonia.

**Phenol-phthalein.**—Dissolve 1 part of the pure commercial product in 200 parts of 50 per cent. alcohol. This indicator cannot be well used with ammonia or in presence of free carbonic acid.

**Methyl orange.**—Dissolve 1 part of the color in 1000 parts of distilled water. Although this solution is very weak a *single drop* is sufficient for an ordinary titration; with more the change of color is less characteristic or sharp. The indicator is valuable in the titration of carbonates or ammoniacal liquids. Carbonic acid does not act on it.

**Rosolic acid.**—Dissolve 1 part in 500 parts of 50 per cent. alcohol. This is a sensitive indicator for the mineral acids.

## Some Volumetric Solutions

**Standard sulphuric acid.**—This is required for the determination of ammonia and for the determination of alkalinity of urine as given in the text. The preparation of the "normal" acid, 49.05 grams of $H_2SO_4$ to the liter, will be given first. As the strongest obtainable commercial acid always contains some water, it is not practicable to weigh out 49.05 grams and dilute it to one liter. The solution may be made accurately by a less direct method, as follows: Measure out 30 cc. of pure strong acid and pour it into 900 cc. of distilled water, with stirring; when the mixture cools dilute it to one liter, which gives a solution a little too strong, and find the actual strength in this manner. Weigh out 2.65 grams of pure, perfectly dry sodium carbonate and dissolve it in a little water, in a beaker. Add two drops of a weak solution of methyl orange as indicator and then from a burette run in some of the sulphuric acid, very slowly, and with constant agitation of the beaker liquid, until the color changes to a faint pink.

At this stage just enough acid has been added to neutralize the carbonate according to the equation:

$$\underset{98.1}{H_2SO_4} + \underset{106}{Na_2CO_3} = \underset{142.1}{Na_2SO_4} + \underset{18}{H_2O} + \underset{44}{CO_2}$$

The 2.65 grams of pure carbonate taken will neutralize 2.4525 grams of pure acid and therefore this weight of acid must be present in the volume added from the burette. It is also the weight of acid which should be present in 50 cc. of the normal solution. If the experiment shows that we have used 48 cc. of the acid in the test with the carbonate it remains to simply dilute with pure water, to bring each 48 cc. up to 50. If we have left 900 cc. we may dilute according to this proportion:

$$48 : 50 :: 900 : x. \quad x = 937.5$$

Therefore, we add 37.5 cc. of water to the 900 cc. to secure the right volume.

From this normal solution a tenth-normal, N/10, may be made by diluting 50 cc. to 500 cc., and any desired solution, below the normal, may be made in a corresponding manner.

**Standard sodium hydroxide solution.**—A fifth normal, N/5, or tenth normal, N/10, alkali solution is employed in measuring the acidity of the urine and in titrating the excess of acid used in the ammonia determination. The preparation of a fifth-normal solution will be given here. This contains 8 grams of actual NaOH to the liter and must be made indirectly as the commercial hydroxide is never of definite strength. A solution of slightly greater than the desired strength is first made and tested, after which water is added in proper amount to bring to the standard dilution. The value of the alkali solution may be found by comparison with the standard sulphuric acid, as described above.

Weigh out 10 grams of good stick sodium hydroxide, dissolve in water, and dilute to one liter. Measure into a beaker, accurately, 50 cc. of tenth-normal sulphuric acid and add a few drops of weak phenol-phthalein solution as indicator. Heat to boiling, and from a burette run into this, gradually,

the alkali solution and continue the addition until a permanent faint red or pink color persists, showing the neutralization of the standard acid by the alkali. If the alkali were of exactly fifth-normal, N/5, strength 25 cc. would be required for this, but as the solution was made a little strong less will be needed. If 23 cc. is sufficient to neutralize 50 cc. of the N/10 acid, each volume of the 23 cc. must be diluted to 25 cc. with water to produce the required solution. 900 cc. of the alkali should be diluted by this proportion:

$$23 : 25 :: 900 : x. \quad x = 978.3$$

Instead of comparing the alkali solution with the standard sulphuric acid a very convenient and accurate method is to compare with potassium bitartrate, "cream of tartar," taken as a standard. This substance may be obtained in a condition of great purity, suitable for weighing out directly. It combines with the alkali according to the equation:

$$\underset{188.1}{KHC_4H_4O_6} + \underset{40}{NaOH} = \underset{210.1}{KNaC_4H_4O_6} + \underset{18}{H_2O}$$

Weigh out 0.9405 gram of the bitartrate, dissolve in 50 to 100 cc. of water, add a little phenol-phthalein, heat to boiling, and run in the alkali solution until the pink color appears. The dilution is made as before. From the equation it appears that the amount of bitartrate taken corresponds to 25 cc. of N/5 solution of alkali.

By diluting 500 cc. of the N/5 alkali solution to 1000 cc. with distilled water a solution of N/10 strength is obtained.

**Standard permanganate solution.**—In the titration of uric acid a solution of potassium permanganate of N/20 strength is employed. As this substance may be obtained in pure condition the standard solution may be made by weighing out, accurately, 1.581 grams, dissolving in distilled water, and diluting to 1 liter. It is customary to control the strength of permanganate solutions by titration with ferrous solutions, but for the purpose of this book that is not necessary.

**Standard silver nitrate solution.**—This is used in the titration of chlorides in urine and is generally of N/10 strength.

Dissolve 16.99 grams of the pure dry crystallized nitrate in distilled water and dilute to 1 liter with distilled water.

This is used in neutral solution with a chromate as indicator. When the Volhard method is employed an acid solution of the nitrate may be very conveniently made by dissolving 10.79 grams of pure silver foil in nitric acid in a flask. The excess of acid is removed by evaporation, a current of air is blown through to drive out nitrous fumes, and then distilled water is added to bring the volume up to 1 liter.

**Standard thiocyanate solution.**—This is employed in N/10 strength in the titration of chlorides by the Volhard method, and in N/50 strength in the titration of uric acid by the Haycraft method. The N/10 solution contains 7.61 grams of the salt in a liter and is made by weighing out about 8 grams of the crystalline commercial product, dissolving to make a liter of solution and then titrating against the standard N/10 silver solution as described in the text. The N/50 solution is made from this by dilution.

**Ferric alum, indicator.**—This is used with the above solutions and is simply a strong solution of ammonium ferric sulphate in water. The salt must be perfectly free from chlorine.

**Standard mercuric nitrate solution.**—This is employed in the titration of urea by the method of Liebig, and is prepared by the process as described in the text.

**Ehrlich's reagent.**—This is applied in the so-called *diazo* reaction and is made in this manner: Dissolve 1 gram of sulphanilic acid in 200 cc. of water with the addition of 10 cc. of pure strong hydrochloric acid. Make a second solution by dissolving 1 gram of sodium nitrite in 200 cc. of water. The reagent for actual use is prepared by mixing 50 cc. of the first solution with 5 cc. of the second. The mixture is added to 50 cc. of the urine to be tested, and then ammonia enough to give a strong alkaline reaction. The sulphanilic acid and nitrite react on each other in this way:

$$C_6H_4.HSO_3.NH_2 + NaNO_2 + HCl = C_6H_4.N_2.SO_3 + NaCl + 2H_2O.$$

The active agent is the diazobenzenesulphonic acid,

$C_6H_4.N_2.SO_3$, which is sometimes employed directly. But, as it is not very stable, it is best prepared in solution by the reaction given.

**Phosphotungstic acid.**—Dissolve 100 grams of crystallized sodium tungstate and 25 grams of glacial phosphoric acid in 500 cc. of water by aid of heat. Add 50 cc. of pure diluted hydrochloric acid.

## Tables Illustrating the Characteristics of Normal and Pathological Urine

### A. Normal Urine

| | |
|---|---|
| **Quantity** | 1000 to 2000 cc. |
| **Color,** | Straw-yellow. |
| **Reaction,** | Acid. |
| **Specific gravity,** | 1.005 to 1.030. |
| **Total solids,** | 16 to 80 grams daily. |
| **Urea,** | 15 to 70 grams daily. |
| **Uric acid,** | 0.2 to 1 gram daily. |
| **Sugar,** | None. |
| **Albumin,** | None. |
| **Diazo reaction,** | Absent or very weak. |
| **Indican,** | Present normally in traces. |
| **Chlorides, NaCl** | 5 to 25 grams daily. |
| **Phosphates,** | 2 to 5 grams of $P_2O_5$. |
| **Sulphates,** | About 2 grams daily. |
| **Deposit,** | Normally slight in fresh urine. |

### B. Clinical Significance

**Quantity.**—Increased largely in diabetes mellitus and diabetes insipidus. Increased also in chronic interstitial nephritis and in amyloid kidney. Decreased in passive hyperemia, acute nephritis, and generally in chronic parenchymatous nephritis.

**Color.**—Light in diabetes mellitus and diabetes insipidus, also in chronic interstitial nephritis, chronic diffuse nephritis and amyloid kidney. Usually darker in chronic parenchymatous nephritis, in passive hyperemia, and acute nephritis. Usually darker in high fever; in jaundice a peculiar greenish yellow. Bloody urine is reddish brown. Many drugs impart color to be recognized only by special tests.

**Reaction.**—Acid normally and in fevers and diabetes. Normal urine becomes alkaline on standing from fermentation. It is usually alkaline in chlorosis and pernicious anemia. Urine may show an ammoniacal reaction from introduction of the ferment into the bladder by a dirty catheter.

**Specific gravity.**—Low in diabetes insipidus and in chronic interstitial nephritis. It is especially high in diabetes mellitus, and high in passive hyperemia and acute nephritis. Large variations naturally follow greatly increased or diminished consumption of liquid.

**Total solids.**—Increased in gross amount in diabetes mellitus and insipidus, but generally diminished in nephritis, chronic and acute, although with scanty discharge the specific gravity may be high.

**Urea.**—In total amount increased in diabetes mellitus and insipidus, but generally diminished in different kinds of nephritis. In acute nephritis and passive hyperemia, with diminished quantity of urine, the *percentage amount* of urea may be increased.

**Uric acid.**—Generally increased in fevers. Bears normally a fairly definite relation to amount of urea, but clinical significance is often obscure.

**Sugar.**—Diabetes mellitus. A small trace of sugar appears to be normally present in urine, but this is not recognizable with the ordinary test. Temporarily, however, sugar may be present without diabetes; it is the continued presence of the substance which is here characteristic and important.

**Albumin.**—Normally absent. Its presence is usually suggestive of disease of kidney. The continued appearance of large amounts of albumin is always pathological. But temporarily, even more than traces may appear without connection with disorders of the kidney, as in the albuminuria of pregnancy, and in albuminuria in which the albumin is derived from parts of the tract below the kidney.

**Globulin.**—Significance in general the same as that of albumin.

**Peptone.**—Generally pathological and suggestive of suppurative changes somewhere.

**Indican.**—Normally present in small amount, but may be greatly increased in diabetes mellitus or in diseases accompanied by marked putrefactive changes in the intestines.

**Diazo reaction.**—Very weak in normal urine but usually sharp in fever urine.

**Chlorides.**—Normally always present, but great variations depend on character of food. May be greatly decreased in pleurisy and acute pneumonia.

**Sulphates.**—Always present in traces. An increase in the ethereal sulphates is pathological, and accompanies intestinal diseases as a result of putrefactive changes.

**Phosphates.**—Normally always present, and variable with diet. Increased in osteomalacia and similar disorders.

**Sediment.**—Normally is slight in fresh urine, but usually becomes abundant, if the urine be allowed to stand, from putrefactive changes. In the following tables the characteristics of certain sediments are given.

### C. Diabetes Mellitus

| | |
|---|---|
| **Quantity,** | Very much increased, may amount to 15 liters, in 24 hours. |
| **Color,** | Pale yellow. |
| **Reaction,** | Acid. |
| **Specific gravity,** | High usually, may reach 1.060. |
| **Total solids,** | Increased in total amount, but lower in percentage. |
| **Urea,** | Increased in total, but percentage low. |
| **Sugar,** | Always present, and from 100 to 900 grams, in 24 hours. |
| **Albumin,** | Usually absent. |
| **Acetone and aceto-acetic acid,** | Usually present. |
| **Indican,** | Usually increased. |
| **Phosphates,** | Increased. |
| **Sulphates,** | Increased. |
| **Deposit,** | Little. |
| **Microscopic examination,** | Negative. |

### D. Diabetes Insipidus

| | |
|---|---|
| **Quantity,** | Greatly increased. |
| **Color,** | Very light, clear. |
| **Reaction,** | Neutral or feebly acid. |
| **Specific gravity,** | Very low. |
| **Total solids,** | Increased in total amount, but low in per cent. |
| **Urea,** | Increased in total amount, percentage low. |
| **Sugar,** | Absent. |
| **Albumin,** | Absent. |

| | |
|---|---|
| **Deposit,** | None. |
| **Microscopic examination,** | Negative. |

### E. Chronic Parenchymatous Nephritis

(Chronic Bright's Disease)

| | |
|---|---|
| **Quantity,** | Usually much below normal. 1000 cc. or less. |
| **Color,** | Dark, sometimes described as smoky, turbid. |
| **Reaction,** | Acid. |
| **Specific gravity,** | Normal or higher. |
| **Urea,** | Below normal. |
| **Albumin,** | Abundant. |
| **Sediment,** | Heavy. |
| **Microscopic examination,** | Hyaline casts, granular casts, epithelial casts, fatty casts, red blood corpuscles, leucocytes, sodium and ammonium urates. |

### F. Acute Nephritis

(Acute Bright's Disease)

| | |
|---|---|
| **Quantity,** | Scanty, often below 500 cc. |
| **Color,** | Dark, smoky, turbid, appearance largely due to blood. |
| **Reaction,** | Usually acid. |
| **Specific gravity,** | High. |
| **Total solids,** | Lessened. |
| **Urea,** | Total quantity much diminished but per cent. high. |
| **Albumin,** | Much present. |
| **Sediment,** | Much. |
| **Microscopic examination,** | Blood corpuscles, leucocytes, blood corpuscle casts, epithelial casts, granular casts, hyaline casts, uric acid, urates, and renal epithelium. |

### G. Chronic Interstitial Nephritis

(Primary Contracted Kidney)

| | |
|---|---|
| **Quantity,** | Usually excessive, 2000 to 4000 cc. frequently. |
| **Color,** | Light. |
| **Reaction,** | Acid. |
| **Specific gravity,** | Low, often 1.005. |
| **Total Solids,** | Diminished. |
| **Urea,** | Much diminished in total quantity and per cent. |
| **Albumin,** | Little or none at all, but under temporary renal congestion it may be considerable. |
| **Sediment,** | Little. |
| **Microscopic examination,** | Hyaline casts, granular casts, but not always present, blood corpuscles absent. |

### H. Chronic Diffuse Nephritis

(Secondary Contracted Kidney)

| | |
|---|---|
| **Quantity,** | Normal or increased. |
| **Color,** | Light and usually clear. |
| **Reaction,** | Acid. |
| **Specific gravity,** | Below normal. |
| **Total solids,** | Diminished. |
| **Urea,** | Low. |
| **Albumin,** | Present, and often in large amount. |
| **Sediment,** | Considerable. |
| **Microscopic examination,** | Casts, numerous and of all kinds, acid urates. |

### I. Amyloid Kidney

(Not Associated with Nephritis)

| | |
|---|---|
| **Quantity,** | Large. |
| **Color,** | Pale yellow, clear. |

| | |
|---|---|
| **Reaction,** | Acid. |
| **Specific gravity,** | Normal or diminished. |
| **Total solids,** | Diminished. |
| **Urea,** | Diminished. |
| **Albumin,** | Usually abundant, accompanied by globulin. |
| **Deposit,** | Absent or small. |
| **Microscopic examination,** | Often negative. A few waxy or hyaline casts may be present. |

### J. Passive Hyperemia

| | |
|---|---|
| **Quantity,** | Scanty. |
| **Color,** | Dark, sometimes bloody in appearance. |
| **Reaction,** | Acid. |
| **Specific gravity,** | High. |
| **Total solids,** | Usually diminished in total amount, but in percentage increased. |
| **Urea,** | Total decreased, but percentage may be increased. |
| **Albumin,** | Present, but variable in amount. |
| **Deposit,** | Generally considerable. |
| **Microscopic examination,** | Hyaline casts, red blood corpuscles, uric acid, and urates. |

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